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International Council for
the Exploration of the Sea

C.M. 1978/E:6
Marine Environmental
Quality Committee

FOURTH REPORT OF THE WORKING GROUP ON
MARINE POLLUTION BASELINE AND MONITORING STUDIES IN THE NORTH ATLANTIC

Charlottenlund, 17-19 May 1978

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1. OPENING REMARKS AND ADOPTION OF AGENDA

The Chairman, Mr. A. Preston, opened the meeting at 9:30 h on 17 May and formally welcomed the members. He noted that since the last meeting, the name of the Working Group had changed, but that the change had not been completely in accordance with the views that the Group had expressed at the last meeting. The agenda was adopted as proposed and is attached as Annex I.

2. MEMBERSHIP OF THE GROUP

As there were a number of new members attending the meeting, each person introduced himself/herself indicating affiliation and fields of primary interest. The list of participants is attached as Annex II. The ICES Environment Officer was appointed Rapporteur.

3. REVISED STRUCTURE OF ICES COMMITTEES, ACMP AND PRESENT STATUS OF THE WORKING GROUP

- 3.1. The General Secretary of ICES informed the Group of the relevant changes in the structure of the Committees and the ACMP which had occurred at the 1977 Statutory Meeting. The major changes were that the Fisheries Improvement Committee had been split into two new committees, the Marine Environmental Quality Committee and the Mariculture Committee. The composition of the ACMP had changed slightly, reducing the number of ex officio members to three and increasing the number of coopted members. There had been no change in the status of the Working Group.
- 3.2. In the discussion, it was noted that the Marine Environmental Quality Committee had a wider area of competence than simply pollution matters. Among others, the Working Group on the Pathology and Diseases of Marine Organisms would report to this committee concerning pollution-related diseases and disorders in fish and shellfish.
- 3.3. The Group then went on to discuss how the subject of marine chemistry should be handled in the ICES structure, a topic which has been under discussion since the 1976 Statutory Meeting. The Chairman of the Sub-Group on Contaminant Levels in Sea Water, Dr. D. Schmidt, reported that the Sub-Group had considered this issue at its recent meeting and, although it felt that ultimately a Committee on Marine Chemistry would be desirable, had recommended the establishment of a working group on marine chemistry as a temporary solution to the problem. In its discussion, the Working Group felt that there were two separate issues involved: (1) the creation of a forum for marine chemists to discuss their own issues, and (2) the development and conduct of specific activities which often require an intergrated approach involving other scientific disciplines. The discussion revealed that many different opinions were held by members, but the Working Group agreed to support the Sub-Group's recommendation in principle. However, it strongly emphasized the importance of ensuring that channels of communication between the proposed marine chemists group and other Working Groups which require chemical input should be

so constructed as to allow speedy and direct contact in both directions. Only in this way was it felt that requests for advice could be made and answered without unnecessary delay.

4. ACTIONS TAKEN BY THE COUNCIL AND ACMP IN THE LIGHT OF THE WORKING GROUP'S THIRD REPORT

- 4.1 The General Secretary reported on the actions taken with respect to those recommendations in the Third Report which would not be discussed under other items of the Agenda. Recommendation 3 regarding the costs of intercalibration exercises had been discussed at the 1977 Statutory Meeting and had resulted in a resolution (C.Res.1977/4:10) requesting the Bureau to "explore the possibilities of financial support for such exercises and report to the Council". The Bureau will consider this matter when it meets on 27-28 June 1978.
- 4.2 It was noted that all the other recommendations of the Working Group had been adopted, in one form or other, by the Council (see C.Res. 1977/1:3, 1:4, 1:5, 2:1, 2:4, 2:16, 2:17, 4:8, and 4:9), and that most of the activities were in progress and some had already been completed.

5. CONSIDERATION OF RELEVANT INTERNATIONAL ACTIVITIES REQUIRING INPUT FROM ICES

5.1. Joint Monitoring Group, Oslo and Paris Conventions

5.1.1. The Environment Officer informed the Group about the results of a meeting of the Joint Monitoring Group (JMG) of the Oslo and Paris Commissions which had been held in the Azores in March 1978. At this meeting, the aims of a joint monitoring program had been discussed and the outline of a program to meet these goals had been drafted. The Environment Officer quoted from the JMG report that

"The JMG had agreed that at least four aims could be established for a joint monitoring program on the basis of the texts of the two conventions, namely (1) the earliest possible assessment of the existing level of marine pollution, (2) the assessment of possible hazards to human health, (3) the assessment of harm to living resources and the marine life (ecosystems), and (4) the assessment of the effectiveness of measures taken for the reduction of marine pollution in the framework of the Conventions".

To achieve these aims, the JMG had agreed to the following methods: (a) for aims (1) and (4), measurements should be taken in water (with and without suspended solids), sediments, and living organisms (trend monitoring) and studies should be done of the input of contaminants from land-based sources in the areas under consideration, (b) for aim (2), measurements should be taken in marine organisms consumed by

man (target monitoring); and (c) for aim (3), measurements should be taken in suitable marine organisms (target monitoring). The JMG had further agreed that the joint program should be based on parts of existing programs which would be harmonized by the use of common procedures for sampling and treatment of samples, intercalibration of the methods of analysis, and use of standard reporting procedures. It had been decided that the program would begin with monitoring the levels of mercury, cadmium, and PCBs in sea water, sediments, and living organisms. Among the organisms to be studied were one species of shellfish (Crangon crangon or Mytilus edulis) and two species of fish (chosen from flounder, plaice, mackerel, and cod).

5.1.2. ICES was requested by the JMG to prepare a manual on the sampling of living organisms, specifying, where necessary, the size, age, part of the organism to be analysed, etc. ICES was also asked to coordinate intercalibration exercises on the analyses of mercury, cadmium, and PCBs in biological materials and sea water. Further discussion of these requests was deferred to the subsequent relevant agenda items.

5.2 GIPME

Dr. Portmann reported on the results of the third meeting of the GIPME Task Team on Marine Pollution Monitoring and Baseline Studies, held in Bergen 25-28 April 1978. Among other activities, the Task Team had approved papers on the Deployment of Analytical Resources in Chemical Residue Monitoring and on the Use of Accumulators in Marine Pollution Monitoring. It had been suggested that a paper on cataclysmic events should be made available to the meeting of the ICES ad hoc Group on Oil Pollution Incidents, in Brest, 7-9 June 1978.

5.3 Open Ocean Programme of IOC/WMO/UNEP

5.3.1. The General Secretary informed the Group that discussions in the ACMP and at the last Statutory Meeting had resulted in a resolution (C.Res.1977/3:1) that the Council officially approach UNEP and IOC in order to explore the best ways and means for cooperation in the preparation of an open ocean monitoring exercise. This approach has been made and a preliminary response has been received from IOC.

5.3.2. In the discussion on this subject, it was pointed out that the intercalibration exercise program being carried out under the Sub-Group on Contaminant Levels in Sea Water (see 8.2) was of direct relevance to the Open Ocean Monitoring Programme because the intercalibration of sampling and analytical methods for determining the levels of trace metals in sea water is an agreed prerequisite to a baseline study or monitoring program in the open ocean.

6. REPORT ON FURTHER DEVELOPMENTS IN RELATION TO
THE INPUT STUDY

- 6.1 The Chairman reminded the Group that at the last meeting a draft report of the Input Study had been presented which was lacking data from Spain and Portugal (who were unable to supply such data) and France, Canada, and the United States (who had agreed to submit data when compiled). This report had been subsequently published as Coop.Res.Rep. No. 77, without the addition of any of the outstanding data. Recently input information had been supplied by the countries who had promised to supply it and the question was how to handle these new data.
- 6.2 The Environment Officer briefly summarized the types of information which had been submitted by France, Canada, and the United States. It was noted that no further information would be available from France and Canada, but the United States hoped to supplement its information with data on the inputs from the Boston area, the Chesapeake Bay and the Delaware River. A report on this additional information will be presented at the 1978 Statutory Meeting.
- 6.3 The Group decided that it would be best to wait for these new data before progressing further, as long as they would be available by the time of the Statutory Meeting. It asked the Environment Officer to prepare a draft report of the extended input study, in consultation with representatives from France, Canada, and the United States, and to present it at the next meeting of the Working Group.
- 6.4 The Group then discussed the general topic of input studies. While it was felt that the work already accomplished was useful and that it had served as a useful stimulus to national authorities, it was agreed that the Regulatory Commissions are in a much better position to collect input data than ICES. The members did, however, emphasise that it is essential that the collection of input data be conducted in an organised and systematic way and that ICES, through this Working Group and the ACMP, had both a legitimate interest and a role to play in the interpretation and planned collection of data. With this in mind, the Working Group felt that its future activities should place much more emphasis on studies designed to develop an understanding of the fate of pollutants in the marine environment. In this context the Working Group considered that estuaries presented a special challenge and it was recalled that the Hydrography Committee had been asked to look into this subject. As no information was available to the Working Group on any action taken by the Hydrography Committee and as the topic was felt to be of relevance to the question of relating inputs to contaminant levels in biota, sediments and sea water the Secretariat was requested to bring the continuing interest of the Working Group to the attention of the Hydrography Committee.
- 6.5 The problem concerning the identification of new substances which may be pollutants was discussed. Dr. Phelps mentioned that several countries have set up a tissue bank which archives tissues from various organisms so that one can trace back the appearance of new substances in the environment which will be detected at some future

time. Dr. Phelps agreed to prepare an information paper on this tissue bank for the next Statutory Meeting. The Group's attention was drawn to the recommendation of the ICES/SCOR Working Group on the study of Pollution of the Baltic, made at its meeting in April, that increased efforts be made on studies of potentially harmful organic substances in the sea, with the aim of identifying these compounds, determining their concentration and persistency and whether their origin is biogenic or anthropogenic. The Working Group endorsed this recommendation, but felt that it should be expanded to specifically include by-products created by specific industrial processes.

7. MATTERS RELATED TO MONITORING USING FISH AND SHELLFISH

7.1 Reports On Regression Analysis Programme

7.1.1. In response to the suggestion made at its 1977 session, the Working Group was informed that four countries (Belgium, Canada, Scotland, and England/Wales) had been able to carry out some investigations into the physiological factors which affect the concentrations of particular pollutants in marine organisms, and had conducted regression analyses on the data obtained. Brief details of the work conducted were provided by the appropriate representatives. Belgium had examined mercury in sole muscle against age, length and weight; Canada had studied the content of α HCH, HCB, DDT, PCB, arsenic, cadmium, copper, mercury, lead, selenium, and zinc in cod liver and muscle, all against length. Scotland had examined mercury in ling and two adjacent 'populations' of dog-fish against age, length and weight. England had examined mercury, copper, and zinc in blue whiting against age, length, and weight on a total population basis and on a sex-separated basis. Similar work had been done on flounder and cod, and in all cases the data had been examined on a wet and dry weight basis. Some work had also been done on organochlorine pesticides and PCBs in flounder liver on a fresh weight and an extracted fat weight basis. These reports are attached as Annex V.

7.1.2. The results of this work indicated that different factors were important for different pollutants and for different species and, on the basis of the two sets of dogfish data, there was evidence of differences for the same pollutant and species with area. In some cases, the differences in pollutant concentration with age, etc., were not large even though the linear correlation was significant. There was some suggestion that, provided fairly tight restrictions were placed on sampling details, the data obtained might be capable of trend analysis, thus confirming the justification of such a practice in the ICES baseline studies. However, it was recognised that such a practice is incompatible with the collection over an extended area and/or time period of samples of fish for human health assessment purposes and that, even if it were not, detection of trends would only be possible if the necessary physiological data were available as well to allow multiple regression analyses to be performed.

7.1.3. It was agreed that the results of the programme of work conducted in the intersessional period were both interesting and valuable and that further similar work should be prosecuted by any country with the available resources. Several countries indicated that they hoped to be able to do such work and that they would report the results either to the forthcoming Statutory Meeting or the next session of the Working Group.

7.1.4. In the light of the work completed so far, it was concluded that it is not possible to make generalisations as to what physiological factors are important or unimportant in affecting the concentration of any particular contaminant in any particular species or area, and that the use of marine organisms in the assessment of risk to human health and for trend analysis represent two different objectives which require two somewhat different approaches to sampling. Thus, in the conduct of monitoring programmes primarily for human health purposes only types of fish used for human consumption should be selected. The Group generally agreed that samples should be analysed on an individual organism basis, although some members felt that analysis of homogenates would be adequate. For each specimen analysed, the parameters of length, weight and age should be determined. This type of programme is straightforward, as the objective is to determine whether there may be any risk to human health in consuming fish and, if so, for which fish from which area.

7.1.5. On the other hand, the Group recognized that there were greater difficulties in using marine organisms in the determination of trends in the level of pollutants in the marine environment, both regarding provision of comparable samples and regarding establishment of whether any observed changes are indeed due to changes in pollutant levels in the environment rather than to the influence of biological variables. Thus, at the outset of any such programme, the Group agreed that it would be advisable to conduct a thorough investigation of the impact of physiological variables on the levels of each contaminant under study in the species of marine organisms and areas to be monitored. For this, it is considered desirable to analyse the samples on an individual organism basis and to collect information on each specimen's length, weight, age, sex, condition factor and liver somatic index. This will permit sequential multiple regression analysis of the data, thereby allowing determination of the effect of the biological variables so studied. On the evidence available so far, it was concluded that such an analysis will have to be done separately for each contaminant and each species, and also according to stock or area, as different variables appear to be significant in different cases. When the importance of each variable has been so established, it will be possible to specify appropriate sampling procedures to be used for the analysis of trends in the levels of contaminants in the species and areas studied. Some members of the Group suggested that only young fish (about 2 years) be used in a trend monitoring programme, in an attempt to decrease or eliminate the possible influence of certain biological factors (e.g., reproductive cycle).

7.2 1977 Coordinated Monitoring Report

7.2.1. The Environment Officer reported that data on contaminant levels in fish and shellfish had been submitted for the agreed areas of the Northeast Atlantic by the United Kingdom, Ireland, Belgium, and the Federal Republic of Germany. Additionally, as agreed at the last meeting, data had been submitted by Canada for the Gulf of St. Lawrence and by the United States for the New York Bight. The U.S. data are for 1978 and will be held for the report on that year. Unfortunately, due to the late submission of most of the 1977 data, there had not been adequate time to prepare a draft of the report for presentation to the Working Group.

7.2.2. The Working Group agreed that the Environment Officer should prepare a draft of the report and distribute it in early summer to those Working Group members who wished to review it, particularly those who had submitted data. Their comments should be sent back by late August so that a final draft could be presented to the ACMP at its meeting during the time of the Statutory Meeting. The Group then agreed that it would approve that a report be prepared in this way, without further formal approval by the Group.

The Group then discussed whether it should continue the Coordinated Monitoring Programme, given that portions of it would overlap with the proposed Joint Monitoring Group programme. The Working Group felt that the Coordinated Monitoring Programme was valuable because it covered measurements of a number of contaminants in biota from areas of interest on both sides of the North Atlantic. It was considered that ICES, with its interests in both preservation of fish resources and protection of the marine environment against the effects of pollution, had a direct need for the continued collection of monitoring data. It was therefore decided that, regardless of any outside customer for the work, the Working Group should continue with its coordinated monitoring activities.

7.3 Proposals for next Intercalibration Programmes and new requests from the Joint Monitoring Group

7.3.1. Heavy metals - The Chairman noted that the reports on the previous series of intercalibration exercises on heavy metals and organochlorines in biological materials had been prepared by Dr Topping and Mr A. V. Holden and had been published as Coop.Res.Rep. No. 80. The Group expressed appreciation to the two coordinators for their work in this area.

7.3.2. A report on the fourth intercomparison exercise was presented by its Coordinator, Dr Topping. This exercise covered analyses of cadmium and lead only, due to the poor performance on these metals in earlier exercises. The same fish flour was used as had been distributed for the third round intercalibration. The results showed a greater level of agreement among the laboratories than has occurred before. The Group suggested that several small amendments be made to the paper and agreed that it should be annexed to this report (Annex VII).

- 7.3.3. Dr Topping then informed the Group about the plans for the fifth intercalibration exercise on analyses of heavy metals in fish material. Young cod from an uncontaminated area will be caught and skinned and the muscle filets will be removed and freeze-dried. Ten percent of the total will be treated for partial removal of mercury. Samples, to be distributed in September, will consist of 20 g of the main sample, 4-5 g of the low-Hg sample, and standard solutions for the metals which are mandatory to be analysed: Hg, Cd, Pb, Cu, Zn, and As. Participants will also be welcome to analyse the following elements on an optional basis: Se, Cr, V, Co, Mn, Ni, Ag, and Fe.
- 7.3.4. This intercalibration will also serve the laboratories which are to take part in the Joint Monitoring Programme. Although they are only required by the JMG to analyse for mercury and cadmium, they will be invited to analyse for the entire suite of metals covered in the exercise.
- 7.3.5. The results of the analyses should be sent to the Coordinator by the end of November 1978, at which time a reminder will be sent out to all laboratories which have not yet submitted their results. Dr Topping suggested and the Working Group subsequently agreed that it was important to emphasise that any data submitted after the final deadline could not be accepted and thus those laboratories would not be considered as having taken part in the intercalibration. The ICES Secretariat was asked to send a schedule of the exercise to the Oslo Commission Secretariat, emphasising the last-mentioned point. The schedule for the organochlorine intercalibration should also be included.
- 7.3.6. Organochlorines - Dr Topping informed the Group of the plans made so far by the Coordinator of the intercalibration exercise on organochlorine residues in biological materials, Mr A. V. Holden. Mr Holden had hoped to distribute standard solutions of the organochlorine residues under study, using hexane as the solvent. However, it had been learned that it is illegal to send inflammable liquids by post or by air. The Group suggested that one possible alternative would be for Mr Holden to specify the type of standards which should be used, their purity (which should be 99%), and the method of preparation of the working standard. The Group also suggested that for the actual samples, fish oil which has not been steam-blown should be used, and spikes should be around 2-3 times background.
- 7.3.7. Dr Topping reported that the distribution scheme will be similar to that used for the fish muscle material. The residues will be the same as those tested in the previous exercise, namely, HCB, α -HCH, β -HCH, γ -HCH, dieldrin, DDE, TDE, DDT, and PCBs. As with the heavy metal intercalibration, the organochlorine intercalibration exercise will also cater for laboratories which will participate in the JMG programme. Although these laboratories are only required to analyse for PCBs for the JMG work they will be invited to analyze for the other organochlorine residues included in the exercise.

7.3.8. The Group then considered whether the policy of identifying each laboratory with its results, which has always been followed in intercalibrations of analyses of contaminants in biological materials, should be continued now that the exercise was to be expanded to include laboratories coming in via the JMG request. The Group agreed that there are several good reasons for ensuring that laboratories are readily identifiable, e.g., so as to allow their performance in the intercalibration exercise to be used in the interpretation of any monitoring data they submit. It was also agreed that the coordinators of the intercalibration exercises should have direct contact with each participant. The question of naming laboratories in any formal publication, while also considered desirable, was viewed as a separate issue and one which could be better dealt with at the time of any proposed publication.

7.4 Extended Baseline Study Report

7.4.1. The Group reviewed a draft report of the extended Baseline Study, which had been prepared by the Environment Officer utilizing data which had been submitted by the United States, Canada, Ireland, and Portugal. The Group agreed that the format of the report was good, but suggested certain changes to the text and tables. It was agreed that a new draft should be prepared by the Environment Officer and circulated as soon as possible to representatives of the countries whose data had been used in the report as well as to any members of the Group who wished to review it. The initial draft would be presented to the ACMP at its mid-term meeting and, if desired, the final draft could be presented at the October ACMP meeting.

7.4.2. Dr. de Barros informed the Group that the results of metal analyses for the Baseline Study could still be expected from Portugal. She also indicated that consideration was being given to the possibility of launching a monitoring programme, at least for organochlorines, at certain sites selected on the basis of the results of the Baseline Study.

7.5 Request from the Joint Monitoring Group for a sampling manual

7.5.1. The Working Group was reminded of the request to ICES from the Joint Monitoring Group for a manual on sampling and pre-treatment of marine organisms for monitoring purposes. The request was phrased in such a way that it was clear that the Joint Monitoring Group expected fairly detailed advice on the sampling criteria for the species of marine organisms selected and the organs to be used for the different pollutants. These should be specified for the two main purposes envisaged in the Joint Monitoring Group's programmes, namely, human health risk assessment and trend assessment.

7.5.2. It was agreed that the information collected in the course of the multiple regression analysis investigations was highly relevant to this question and in fact indicated that the problem was not as simple as may have been envisaged. After some discussion, the Group agreed that, bearing in mind the size of the area and the range of pollutants and species, generalisations of the type expected could not be made. It was, however, agreed that detailed advice could be

given on the necessary steps to be taken for sample collection and preparation for analysis and also that guidance could be included on what data would be necessary to allow trend analysis to be performed.

7.5.3. It was noted that the Joint Monitoring Group had, as an interim measure, suggested the adoption of the ICES North Atlantic sampling protocol and it was therefore agreed that this should be used as the basis of the Sampling Manual to be prepared. It was also noted that the Baltic Group had prepared a manual for a similar purpose and that some sections should be adopted for North Atlantic purposes. The Working Group also agreed that, as similar changes would need to be made for the purpose of guiding participants in the ICES coordinated monitoring programme, the same manual should be used for both purposes. However, in recognition of the slightly different scope of the ICES coordinated monitoring in terms of pollutants, species and areas, it was agreed that a few additional instructions should be included as footnotes for the use of ICES participants only.

7.5.4. A series of changes in the sampling protocol were discussed and the Environment Officer was charged with producing a draft manual incorporating these changes. The draft should be circulated to all members of the fish Sub-Group for comment, due within one month of circulation. The draft should then be submitted to ACMP for approval subject to any changes to be suggested by the fish Sub-Group members.

8. MATTERS RELATED TO SEA WATER ANALYSIS

8.1. Report from Dr Schmidt's Sub-Group

8.1.1. Dr D. Schmidt, Chairman of the Sub-Group on Contaminant Levels in Sea Water, introduced the report of the third meeting of the Sub-Group (ICES, C.M.1978/E:5), which had been held in Charlottenlund the previous week. He reviewed briefly the progress on the programme of intercalibration exercises and informed the Group of the Sub-Group's recommendations regarding the handling of the JMG request for intercalibration exercises on analyses of mercury, cadmium, and PCBs in sea water (see Section 8.2). The Sub-Group had also been approached by the Chairman of the IOC/GIPME Group of Experts on Methods, Standards, and Intercalibration (GEMSI) regarding coordinating an intercalibration exercise for analyses of trace metals in sea water which would include interested non-ICES IOC countries. GEMSI had suggested that ICES continue in its lead role in the intercalibration of trace metal analyses in sea water, whereas GEMSI would concentrate its work on organochlorine analyses in sea water.

8.1.2. Dr Schmidt also reported that the Sub-Group had considered a draft report, prepared by a small drafting group, which surveyed national reports submitted in response to an ICES request and other published reports on studies of trace metal levels in open ocean waters and indicated the state of the art of these programmes. The main conclusion of this report was that there were not many data available for open ocean areas and what were available were not comparable due to differences in sampling methods, handling, and analysis.

8.1.3. The Group expressed great interest in this draft report on trace metal "baseline studies" in open ocean areas and felt that it would be very useful to include information on trace metal studies in near-shore areas. Although it was recognized that the lack of comparability of data presented a significant problem, the Group felt that by a careful consideration and evaluation of all the data available, a very broad comparison could be made between the levels of trace metals in open ocean waters and those in coastal waters. Dr Topping, speaking for the drafting group responsible for the original document, agreed that it would be possible to extend this work to near-shore areas and to draw such a broad comparison. The Working Group looked forward to receiving this expanded report.

8.1.4. The Group then took up the issue of the GEMSI suggestion that ICES continue in its lead role in trace metal intercalibrations in sea water and extend the possibility of participation to other IOC countries outside ICES. The Group felt that this did not present a problem as long as it did not involve an ICES commitment to further work on the IOC/UNEP/WMO open ocean monitoring programme beyond the intercalibration phase. Thus, the Working Group supported the suggestion that ICES continue its lead role in the coordination of intercalibration exercises for trace metals in sea water and that participation be opened to appropriate laboratories from non-ICES countries, provided that this can be done without cost to ICES.

8.1.5. Regarding the portion of the GEMSI suggestion dealing with recognition of GEMSI in a lead role in the intercalibration of organochlorine analyses in sea water, the Group agreed that it was important to avoid duplication of effort on this type of programme but also felt that ICES would not wish to abrogate any responsibility it might have in this field, especially in respect of future needs (cf. JMG request in relation to PCBs). However, the Group saw no immediate need to become involved in this area.

8.1.6. The Working Group then reviewed the list of recommendations which the Sub-Group had agreed to (see ICES Doc. C.M.1978/E:5, Annex III) and made the following decisions:-

Recommendation 1 (to extend the terms of reference of the Sub-Group to include all chemical contaminants in sea water) - the Group did not support this because it felt that only trace metals would be studied at the present time.

Recommendation 2 (regarding submission of a statement on approaches to monitoring to the JMG as part of ACMP advice) - supported for transmission to ACMP.

Recommendation 3 (regarding publication of the review of trace metal studies in open ocean waters) - supported in principle, but with additions as suggested (see 8.1.3.).

Recommendation 4 - The Group supported the portion of the recommendation dealing with ICES responsibility for international intercalibration for trace metals in sea water, subject to approval by ACMP and the Council. Regarding the second portion (the GEMSI role in intercalibration of organochlorine analyses in sea water), the Group felt that although it did not intend to do any work on this subject

in the immediate future it could not recommend the abrogation of ICES responsibilities in this area.

Recommendation 5 (on the establishment of a Working Group on Marine Chemistry) - supported with qualifications.

Recommendation 6 (on the next meeting of the Sub-Group) - supported.

8.1.7. Several members of the Working Group expressed interest in being informed of the activities of the Working Group on Eutrophication, which had been conducting studies of the levels of nutrients in the North Sea. However, upon looking into this matter, it was discovered that this Group had been dissolved at the end of 1976.

8.2. Intercalibration - Progress with Report on Work up to 1977 and Present Status

8.2.1. Dr Schmidt reported that the results of the intercalibration exercise on mercury in sea water had been published in Marine Chemistry (6:87-95(1978)) and that there had been considerable international interest in these results. The reports on the first and third intercalibrations of analyses of other trace metals in sea water have been recommended for publication (C.Res.1977/1:5) but the manuscript is not expected to be ready before the end of the year.

8.2.2. The fourth intercalibration exercise, coordinated by Dr Bowers, is now well underway and many of the samples have been distributed. The results are expected to be available for presentation at the next meeting of the Working Group. Planning is also underway for the fifth intercalibration, a one-ship exercise intended to investigate the effects of different sampling methods and devices and shipboard sample pretreatment on the results ultimately obtained.

8.3. New Request from Joint Monitoring Group

8.3.1. Dr Schmidt reported that the Sub-Group had carefully considered the JMG request for ICES to conduct intercalibration exercises on analyses of mercury, cadmium, and PCBs in sea water and had concluded that a positive response should be made to this request. For mercury and cadmium, the Sub-Group had already obtained sufficient experience from earlier exercises that it was able to design two intercalibration exercises (one for mercury and the other for cadmium and additional metals, if desired) and the next step was to find a coordinator for each experiment.

8.3.2. The Working Group supported the Sub-Group's decision on the mercury and cadmium intercalibrations and went on to discuss the issue of the expenses the coordinators of these exercises will incur. It was decided that because the request for these exercises comes from an organization outside ICES, the future coordinators should prepare estimates of the total costs which would be incurred in the conduct of the exercise, itemized according to materials, shipping

costs, manpower, ship time etc. The Group hoped that the persons proposed by the Sub-Group to act as coordinators, Mr Ólafsson and Dr Thibaud, would be able to inform the ACMP at its mid-term meeting whether they will be able to coordinate an exercise and, if so, to estimate the costs which would be involved.

8.3.3. Regarding the request for coordinating an intercalibration of PCB analyses in sea water, the Sub-Group had also recommended a positive response and had felt that the best way forward was for ICES to cooperate with GEMSI in further developing analytical techniques for organochlorine determinations and eventually in conducting intercalibration exercises. The Working Group did not concur with this course of action but felt instead that it should advise the ACMP that very few laboratories would be capable of conducting meaningful work in this area. Recognising that the work proposed by GEMSI might eventually lead at least to a reasonably feasible method of analysis, it was suggested that the ACMP should inform the JMG of the present situation and the fact that they would keep the question under review and, should an intercalibration exercise subsequently seem appropriate, advise the JMG accordingly.

9. REPORTS ON BIOLOGICAL EFFECTS STUDIES

9.1. Progress in Organization of Proposed Workshop

9.1.1. Dr McIntyre, Convenor of the proposed Workshop/Symposium on the problems of monitoring the biological effects of marine pollution, reminded the Group that this proposed Workshop is a result of the work of the ad hoc Group on the Feasibility of Effects Monitoring. He noted that the report of this ad hoc Group had been recently published as Coop.Res.Rep. No. 75. The plans for the Workshop are being made by a small steering group, which has drawn up a list of persons with relevant experience who should be invited to participate. This group is exploring the possibilities for holding the Symposium at Duke University Marine Laboratory, Beaufort, North Carolina, from 26 February to 2 March 1979. Financial support is anticipated from the United States.

9.1.2. Dr McIntyre went on to inform the Group of the establishment of a GESAMP group on biological effects of marine pollution, of which he is chairman. He read the terms of reference of this group, which are very similar to those of the ICES ad hoc group and to the aims of the Workshop.

9.2. Reports on Pilot Studies Conducted Unilaterally by Member Countries

9.2.1. Dr Pearce informed the Group of the U.S. National Oceanic and Atmospheric Administration/National Marine Fisheries Service programme, OCEANPULSE. This programme is an interdisciplinary study of the possible man-induced changes in the marine environment and its living resources in the coastal waters off the northeastern United States. The first cruise has just occurred and Dr Pearce indicated that they hoped to have several cruises per year in the future over

a fairly long period of study, perhaps ten years. Other U.S. institutes are assisting in the work and some foreign scientists will also participate.

9.2.2. Dr McIntyre noted that a Dutch study of vertebral deformities had been presented at the last Statutory Meeting (ICES C.M. Doc.1977/E:5) and that at this Meeting, the Council had formulated a request (C.Res. 1977/4:11) that pollution-related diseases in marine organisms be studied and their incidence reported to ICES. In this connection, the Working Group expressed interest in the activities of the Working Group on Pathology and Diseases of Marine Organisms on pollution-related diseases. Not only would it be of value to receive reports of this latter Working Group, but a useful input to its work could be made by establishing mutual contacts. The ICES Secretariat was asked to assist in establishing these contacts.

9.2.3. Dr Phelps reported on the Mussel Watch programme in the U.S. as well as on a programme in his laboratory which is studying whether it is possible to work with a reference population of Mytilus. It was also reported that a Mussel Watch programme has been started on the south coast of Iceland, while Scotland has recently completed such a programme for much of its coastline, studying organochlorines and heavy metals. An English/Welsh study, which had been started in the Channel and is now being extended to the entire coastline, will study levels in mussels of heavy metals, organochlorines, and specific compounds of probable petroleum origin and particular petroleum fractions.

9.2.4. The Group noted that there will be a special session of the Marine Environmental Quality Committee at the next Statutory Meeting covering the results of the Mussel Watch and related programmes; it will probably be expanded to include the use of marine organisms in monitoring programmes in general.

10. REPORT ON MATTERS RELATED TO SEDIMENT STUDIES

10.1. Progress with Sediment Symposium under Dr Postma

10.1.1. The Chairman reminded the Group that, as a result of the report of the ad hoc Meeting of Specialists in the Field of Pollutants in Sediments presented at the last meeting, it had been suggested that a Symposium be held to discuss the issues concerned with pollutant interchanges in the sediments and relating these to interchanges with suspended sediments and the water column. The ACMP had expanded the Working Group's recommendation on this subject to ask that the Symposium develop the outline plans for a pilot study of pollutant interchanges in the sediments. The Chairman had contacted Dr Postma, Chairman of the ad hoc Meeting and Convenor of the Symposium, and conveyed to him the appreciation of the Working Group for his work and also the suggestions which had been made with respect to the proposed Symposium.

10.1.2. Dr Postma has subsequently proposed that this Symposium be held in connection with a meeting of the International Association of Sedimentologists, which will be held in Texel in September 1979. The details of the Symposium have yet to be worked out.

10.1.3. The Group welcomed this news of the proposed Symposium and expressed the hope that it will be attended by specialists representing a broad spectrum of fields associated with pollutants in sediments so that the current state of knowledge can be adequately reviewed and utilized in planning the pilot study.

10.2. Report on Pilot Studies Conducted by Member Countries

It was reported that Canada is conducting a large sediment study in the St. Lawrence estuary. The United Kingdom has been studying sediments in some dumping grounds. In Portugal, three studies had been started since the last Working Group meeting. The use of sediments as an indicator of petroleum hydrocarbon pollution is under study on the coast of Greenland. In the Federal Republic of Germany, pilot sediment studies begun in 1970 have led to official monitoring programmes on sediments in the German Bight and the southern Baltic. Samples are taken once per year at a large number of stations and preserved, with some samples receiving detailed analyses.

11. PETROLEUM HYDROCARBONS

11.1. Ecofisk meeting

Dr McIntyre reported that after the blow-out on the Bravo platform in the Ecofisk field last year, studies of the impact of the oil on the surrounding environment had been conducted by Norwegian scientists with the assistance of scientists from other countries. A final meeting of persons involved in these studies had been held in Norway early this year to discuss the results of their work and produce a draft of a book presenting them. This book was now under review and it was hoped that it would be printed in hard-cover form in Autumn 1978.

11.2. Anglo-Norwegian Programme and Intercalibration

11.2.1. Dr Palmork reported on an intercalibration programme presently under way between his laboratory and the MAFF Laboratory at Burnham-on-Crouch, both of which use the same type of equipment and methodology. Four samples are being analysed in the intercalibration: one of Ecofisk crude oil, and three water samples containing high, medium, and low concentrations of oil. The problems involved in including more laboratories in such a programme were discussed. Among these were mentioned the fact that oil contains so many different compounds, the laboratories must agree on which specific compounds to look for; also some of the petroleum components in sea water change rapidly with time, so there is a problem of comparability of intercalibration samples.

11.2.2. The Group expressed considerable interest in this programme and there was some mention of the desirability of setting up a sub-group dealing with analytical techniques and intercalibration of analyses for petroleum hydrocarbons. However, it was decided that the actual level of support for such a sub-group should be determined first. Thus, the Working Group asked Dr Palmork and his English collaborator

to present a paper to the Marine Environmental Quality Committee at the next Statutory Meeting describing what has been done so far in the bilateral programme and commenting on the advisability of setting up a sub-group on the subject of petroleum hydrocarbon analysis in sea water. Based on the interest in and response to this paper, the Group could decide what step to take next.

12. CONSIDERATION OF FUTURE ROLE AND ACTIVITIES OF THE WORKING GROUP

The Chairman informed the Group that at the last mid-term meeting of ACMP a small sub-group had been set up under his chairmanship to draft a document on monitoring strategy for discussion at the ACMP meeting next month. He presented this draft document to the Group for their comments and suggestions. The Group discussed the document and gave several ideas for changes and additions, which the Chairman agreed to consider when he redrafted the paper.

13. CONFIRMATION OF DEADLINES AND APPROVAL OF RECOMMENDATIONS

The Group reviewed the list of activities which had been agreed upon during the meeting (see Annex III) and approved several recommendations (see Annex IV).

14. DATE OF NEXT MEETING

The Group recommended to hold its next meeting in May 1979 for four days.

15. ANY OTHER BUSINESS

The Chairman informed the Group that the ACMP was interested in its views regarding the definition of marine pollution and the use of marine organisms to determine the level of pollution. The Group decided that, as much of this topic was expected to be discussed at the next meeting of the Marine Environmental Quality Committee and at the proposed Workshop on Effects Monitoring, it would defer consideration of this topic for the time being.

16. CLOSURE OF MEETING

The Chairman thanked the participants for their contributions to the meeting and closed it at 16.00 hrs on 19 May.

Annex I

AGENDA

Working Group on Marine Pollution Baseline and Monitoring Studies
in the North Atlantic

Charlottenlund Slot, 17-19 May 1978

1. Opening remarks and adoption of Agenda
2. Membership of the Group
3. Revised structure of ICES Committees, ACMP and present status of the Working Group
4. Actions taken by the Council and ACMP in the light of the Working Group's Third Report
5. Consideration of relevant international activities requiring input from ICES:-
 - 1) Joint Monitoring Group, Oslo and Paris Conventions
 - 2) GIPME
 - 3) Open Ocean programme of IOC/UNEP/WMO
6. Report on further developments in relation to the Input Study
7. Matters related to monitoring using fish and shellfish:-
 - 1) Reports on regression analysis programme
 - 2) 1977 Coordinated Monitoring Report
 - 3) Proposals for next Intercalibration Programmes and new requests from the Joint Monitoring Group.
 - 4) Extended Baseline Study Report - USA, Canada, Ireland, and Portugal
 - 5) Requests from Joint Monitoring Group for a Sampling Manual
8. Matters related to Sea Water Analysis:-
 - 1) Report from Dr Schmidt's Sub-Group
 - 2) Intercalibration - progress with report on work up to 1977
- present status
 - 3) New request from Joint Monitoring Group

9. Reports on Biological Effects Studies:-
 - 1) Progress in organisation of proposed Workshop
 - 2) Reports on pilot studies conducted unilaterally by member countries
10. Reports on matters related to sediment studies
 - 1) Progress with Sediment Symposium under Dr Postma
 - 2) Reports on pilot studies conducted by member countries
11. Petroleum hydrocarbons:-
 - 1) Ekofisk meeting
 - 2) Anglo-Norwegian programme and intercalibration
12. Consideration of future role and activities of the Working Group
13. Confirmation of deadlines and approval of recommendations
14. Date of next meeting
15. Any other business
16. Closure of meeting

Annex II

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Annex III

ACTION LIST

1. All members participating in the 1978 Coordinated Monitoring Programme to send their data to the Environment Officer, with a copy to the Chairman of the Fish Sub-Group, by 31 March 1979.
2. Dr Pearce to prepare a report on additional inputs from the United States for presentation to the 1978 Statutory Meeting.
3. The Environment Officer to prepare an extended input study report, in consultation with representatives from Canada, France, Greenland, and the United States, for presentation at the next Working Group meeting.
4. All members to promote studies on the transport and fate of pollutants in the estuarine environment and report on progress at the next meeting.
5. Dr Phelps, with assistance from Mr Holden, to prepare an information paper on the tissue bank for presentation to the 1978 Statutory Meeting.
6. All members to report on the identification of new, possibly polluting, substances found in the marine environment and Dr Portmann to prepare paper on GESAMP activities in this field for the next meeting.
7. All members, who have the possibility, to begin or continue studies involving multiple regression analyses comparing contaminant levels in selected species of fish with appropriate physiological parameters.
8. The Environment Officer to draft a report on the 1977 Coordinated Monitoring Programme, circulate it in early summer to interested members, and present the next draft to ACMP in October.
9. All members wishing to participate in the heavy metal intercalibration on fish material to so notify Dr Topping; those wishing to participate in the organochlorine intercalibration to notify Mr Holden.
10. The Environment Officer to prepare a second draft of the extended baseline study, obtain review by interested members, and present to ACMP.
11. Drs Topping, Bewers, Kremling, and Jones to amend their draft report reviewing studies of trace metal levels in open ocean waters of the North Atlantic by including appropriate studies of trace metals in near-shore waters, for presentation at the next Working Group meeting.
12. Mr Ólafsson and Dr Y. Thibaud to try to inform ACMP by the time of its mid-term meeting whether they are able to coordinate an intercalibration exercise on mercury or cadmium, respectively, in sea water and, if so, to provide the ACMP with an itemized estimate of expected costs.

13. The ICES Secretariat to inform the Working Group on the Pathology and Diseases of Marine Organisms of the interest of the Working Group in their work and its desire to be represented at their next meeting.
14. Dr Palmork, with the assistance of his English collaborator, to prepare a paper on the Anglo-Norwegian work on petroleum hydrocarbons for presentation at the 1978 Statutory Meeting, with a view to determining the interest in and support for a sub-group on petroleum hydrocarbon analyses.

Annex IV

RECOMMENDATIONS

Recommendation 1

The Working Group recommends that, with reference to C.Res. 1977/4:9 (ii), the Council be asked to approve the plans for the conduct of a fifth intercalibration exercise on trace metal analysis in sea water.

Recommendation 2

The Working Group recommends that the report on the extended baseline study in the North Atlantic and the report on the extension to the Input Study be published in the ICES Cooperative Research Report series, possibly as a single document.

Recommendation 3

The Working Group supports the recommendation of the ICES/SCOR Working Group on the Study of the Pollution of the Baltic (ICES Doc.C.M.1978/E:4, Rec. 2) that all member countries study and report the appearance of new, possibly harmful, substances in the marine environment, and further recommends that this be expanded to include the by-products of industrial processes.

Recommendation 4

The Working Group recommends that its next meeting be held in May 1979 for four days.

ANNEX V

REPORTS

on

REGRESSION ANALYSIS PROGRAMME

CANADA (by G. R. Sirota)

Regression Analysis of Heavy Metal and Organochlorine Residue
Concentrations in a Statistically Sampled Population of Atlantic
Cod (*Gadus morhua*)

Following the sampling protocol as presented at the 1977 meeting (Annex IV) preliminary statistical analysis has shown several relationships of high significance value.

Enclosed are "F" values calculated from r^2 (coefficient of determination) data for organochlorine and heavy metal residues in cod liver and muscle. A table of theoretical F values for significance levels from 75% to 99.9% is also listed. As can be seen from the calculated F values, there are very highly significant relationships between length, and the concentration of PCB, α -HCH, HCB, and pp-DDT in cod liver.

F values for heavy metal residues range from very highly significant (length vs. Arsenic concentration in cod liver) to very low significance level (length vs. lead concentration in cod liver).

This is a preliminary statistical analysis, a more detailed analysis (in progress) will be summarized and sent to you when it is completed.

PRELIMINARY STATISTICAL ANALYSIS : ICES 1977 DATA

(metal residues : cod liver)

Significance of Relationship

F values for n = 37

<u>Significance level</u>	<u>F</u>
75 %	1.37
90 %	2.86
95 %	4.11
97.5 %	5.49
99 %	7.39
99.5 %	9.01
99.9 %	13.1

F values below calculated from fit of log x and log y to $y = a + bx$, ie. $\log y = a + b \log x$

where x = length (cm.)
y = concentration of contaminant

$$F = \frac{(n-2) r^2}{1-r^2} \quad r^2 = \text{coefficient of determination}$$

<u>Relationship</u>	(Log-Log)	<u>F calculated</u>	<u>r</u>
Length vs. As		37	0.65
Length vs. Cd		20	0.47
Length vs. Cu		15	0.48
Length vs. Hg		3	0.24
Length vs. Pb		0.0013	0.01
Length vs. Se		0.62	0.11
Length vs. Zn		0.37	0.09

PRELIMINARY STATISTICAL ANALYSIS : ICES 1977 DATA

(metal residues : cod muscle)

Significance of Relationship

F values for n = 52

<u>Significance level</u>	<u>F</u>
75 %	1.36
90 %	2.84
95 %	4.03
97.5 %	5.42
99 %	7.17
99.5 %	8.83
99.9 %	12.29

F values below calculated from fit of Log x and Log y to $y = a + bx$, ie. $\log y = a + b \log x$

where x = length (cm.)
y = concentration of contaminant

$$F = \frac{(n-2) r^2}{1-r^2} \quad r^2 = \text{coefficient of determination}$$

<u>Relationship (Log-Log)</u>	<u>F calculated</u>	<u>r</u>
Length vs. As	8.7	0.39
Length vs. Cd	13	0.46
Length vs. Cu	1.4	0.16
Length vs. Hg	0.08	0.04
Length vs. Pb	7.2	0.36
Length vs. Se	1.8	0.19
Length vs. Zn	0.05	0.03

PRELIMINARY STATISTICAL ANALYSIS : ICES 1977 DATA

(organochlorine residues)
cod muscle & liver

Significance of Relationship

F values for n = 52

<u>Significance Level</u>	<u>F</u>
75%	1.36
90%	2.84
95%	4.03
97.5%	5.42
99%	7.17
99.5%	8.83
99.9%	12.29

F values below calculated from fit of Log x and Log y
to $y = a + bx$, ie. $\log y = a + b \log x$

where x = length (cm.)
y = concentration of contaminant

$$F = \frac{(n-2) r^2}{1-r^2} \quad r^2 = \text{coefficient of determination}$$

	<u>Relationship</u>	(Log-Log)	<u>F calculated</u>	<u>r</u>
Liver	Length vs. PCB		71	0.77
	Length vs. α -HCH		33	0.63
	Length vs. HCB		23	0.56
	Length vs. pp ¹ -DDT		35	0.64
Muscle	Length vs. PCB		6.2	0.34

ICES WORKING GROUP ON
MARINE POLLUTION BASELINE AND MONITORING STUDIES IN THE NORTH ATLANTIC
Results of the Regression Analysis Studies, Scotland

G. Topping
DAFS, Marine Laboratory, Aberdeen
Scotland

1) COMMON LING

During 1976 samples of common ling were obtained from a number of areas around the North Sea. The fish were aged, measured and weighed prior to being analysed for their mercury content. The sample of 52 fish ranged in age from 5+ to 16+, in length from 50 to 140 cm and in weight from 600 to 14 800 g.

Concentrations of mercury in the muscle tissue were plotted against age, length, and weight (Figs. 1, 2, and 3). It appeared that mercury concentration increased with age and with weight and, as would be expected from the following weight/length relationship

$$w = 0.002244 e^{3.1687} \quad \text{where } w = \text{weight in g.}$$
$$e = \text{length in cm}$$

with some power of length.

On the basis of these results, therefore, it was decided to fit a relationship of the form

$$y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 \dots \dots \dots (1)$$

where $y =$ mercury concentration ($\mu\text{g.g}^{-1}$ wet weight)

$x_1 =$ age (years)

$$x_2 = 0.002244 e^{3.1687}$$

$x_3 = \frac{w - \hat{w}}{\hat{w}}$ (\hat{w} , the predicted weight on the basis of length)
(relative difference between observed and predicted weight)

The variable x_2 was chosen to satisfy the power relationship between mercury concentration and length, and since weight is to some extent now redundant, being directly related to x_2 , x_3 was chosen to possibly explain any residual variability in concentration due to fish being either above or below the average weight of a fish of a given length.

A multiple regression analysis was carried out, and the relationship (1) above was found to explain 74.4% of the total variability in mercury concentrations. However, the variable x_2 alone was found to explain 74.4% of the total variability. The high correlations between the variables indicate that all of them acting together are no more effective than the best of them acting alone. The relationship (1) was therefore reduced to

$$y = 0.05392 + 0.00002123 x_2 \dots\dots\dots(2)$$

On the basis of the above it was decided to fit an empirical relationship of the form

$$y = a (\text{length})^b$$

this resulted in the following relationship

$$y = 0.00002766 (\text{length})^{1.8826} \dots\dots\dots(3)$$

which suggest that

mercury concentration \propto square of the length of the fish.

The relationship in (3) was found to explain 81.7% of the total variability. In statistical terms both relationships (2) and (3) are equally good.

2) DOGFISH

During the first two months of 1978 samples of dogfish were collected from the North and South Minch, west of Scotland. The samples were sexed, aged, and measured prior to being analysed for their mercury content.

The results of this investigation can be summarised as follows:-

Frequencies of Mercury Concentration for Males and Females

Mercury Concentration ($\mu\text{g}\cdot\text{g}^{-1}$)	North Minch		South Minch	
	males	females	males	females
0.00-0.04				2
0.05-0.09	2		3	5
0.10-0.14	7		6	3
0.15-0.19	7	4	5	
0.20-0.24	7	5	4	
0.25-0.29	6	1	4	1
0.30-0.34	1	2		1
0.35-0.39	1	1	1	1
0.40-0.44	1			
0.45-0.49	1		2	
0.50-0.54	1			2
≥ 0.55				1

Mean Lengths (cm) and Length Ranges of Fish

	Males		Females	
	mean	range	mean	range
North Minch	77.8	68-88	97.2	91-105
South Minch	75.4	65-88	84.4	60-108

It was decided to fit the following relationship for each area:-

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3$$

where $y =$ mercury concentrations ($\mu\text{g}\cdot\text{g}^{-1}$)

$x_1 =$ age (years)

$x_2 =$ length (cm)

$x_3 =$ sex (1 for males, 0 for females)

North Minch

Only age appeared to contribute significantly to the relationship which resulted in the following equation:-

$$y = 0.073 + 0.00859x_1 \dots \dots \dots (1)$$

South Minch

Both age and length contributed significantly to the relationship which resulted in the following equation:-

$$y = -0.342 + 0.01512x_1 + 0.00375x_2 \dots \dots \dots (2)$$

As the inclusion of length was just statistically significant, it was decided to adjust this relationship to eliminate the length variable in order to obtain a direct comparison of the data from the South Minch with that of the North Minch. The linear relationship between mercury concentration and age alone was found to be:-

$$y = -0.102 + 0.01842x_1 \dots \dots \dots (3)$$

Comparison of Data from South and North Minch

Although the constant terms in (1) and (3) did not differ significantly, there was a statistically significant difference between the slopes for each regression.

Both equations (1) and (3) may be simplified further, so that the regression lines pass through the origin. The equations fitted in this way are:-

North Minch:- Mercury concentration = 0.01248 age

South Minch:- Mercury concentration = 0.01305 age

SUMMARY

- 1) The mercury content of ling was found to be directly related to the square of the length of the fish.
- 2) The mercury content of dogfish was found to be directly related to the age of the fish.
- 3) On the basis of this survey of dogfish it appears that there could be significant differences between the two areas investigated.

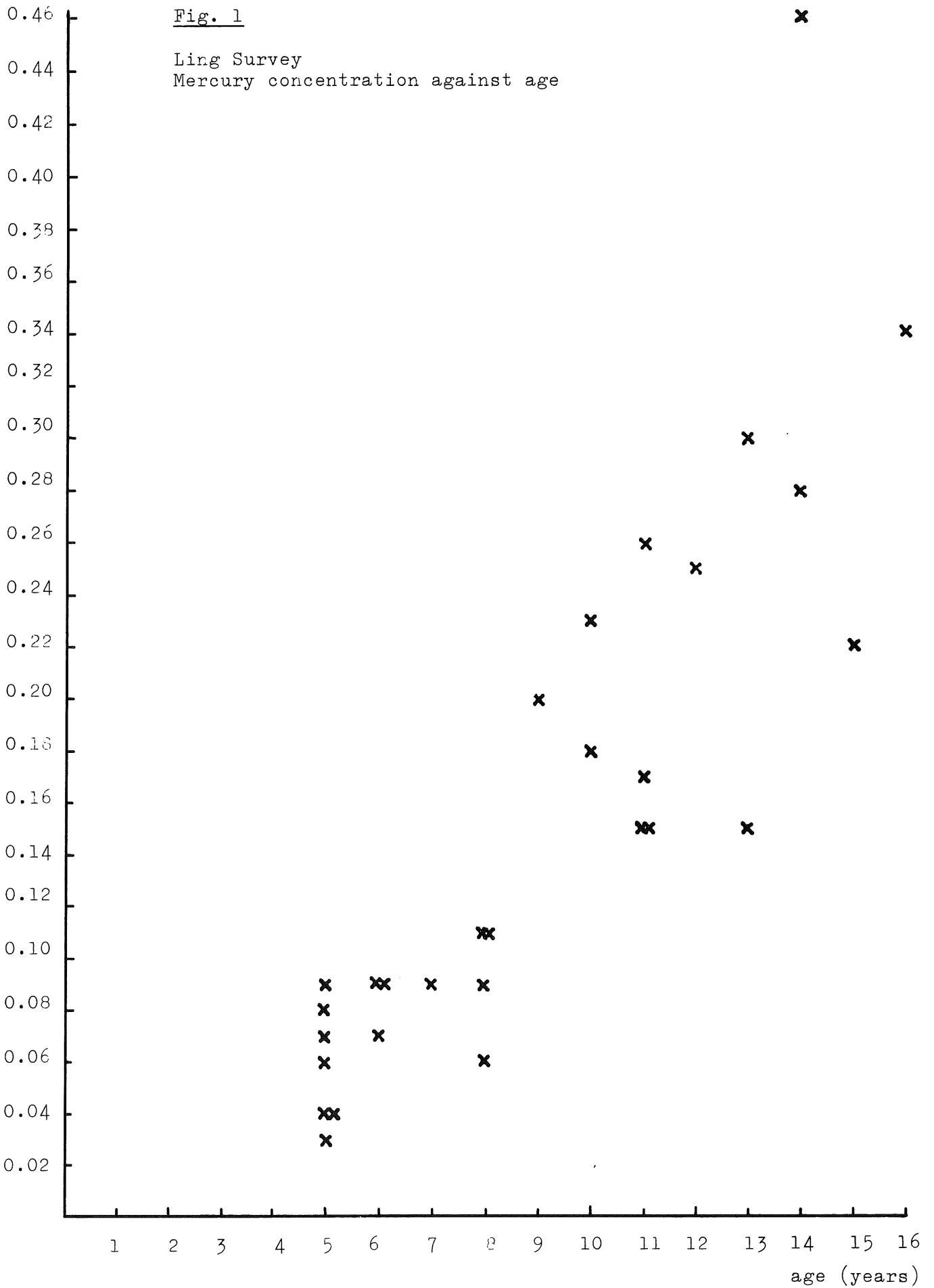
CONCLUSION

In the context of monitoring, it is advisable that studies of this kind should be conducted on each selected fish species.

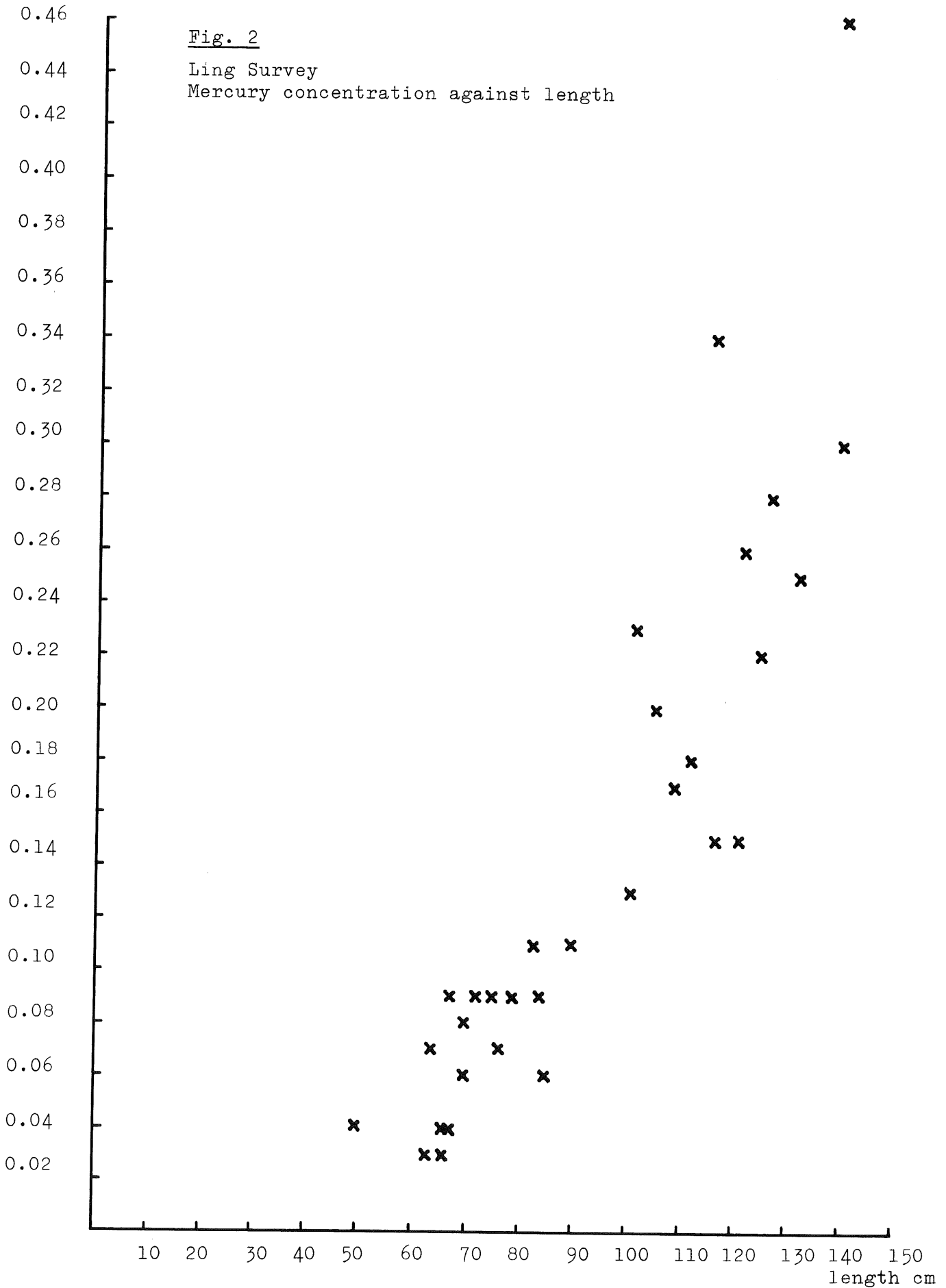
Hg conc.
 $\mu\text{g.g}^{-1}$ (wet weight)

Fig. 1

Ling Survey
Mercury concentration against age



Hg conc.
 $\mu\text{g.g}^{-1}$ (wet weight)

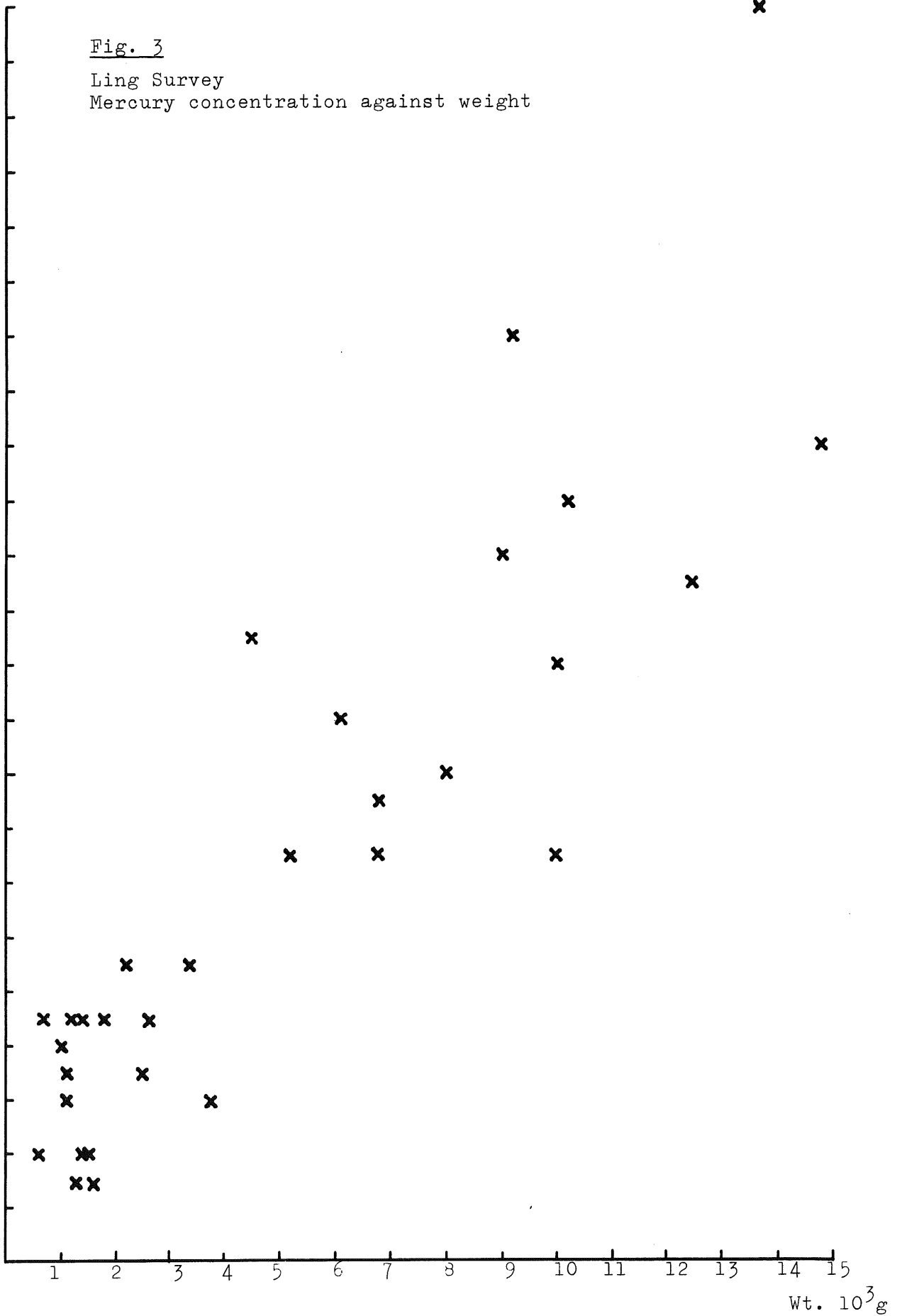


Hg. conc.
 $\mu\text{g}\cdot\text{g}^{-1}$ (wet weight)

0.46
0.44
0.42
0.40
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02

Fig. 3

Ling Survey
Mercury concentration against weight



ICES WORKING GROUP ON

MARINE POLLUTION BASELINE AND MONITORING STUDIES IN THE NORTH ATLANTIC

Results of the Regression Analysis Studies, Belgium

Dr. W. Vyncke

Regression between mercury content and length, weight, and age of sole (*Solea solea*) from the North Sea, the Irish Sea and the Bristol Channel (*)

In 1973, the mercury content of 120 soles from each of three fishing grounds (North Sea, Irish Sea and Bristol Channel) was determined. The fishing area of origin is mentioned in figure 1. There was no significant difference between the two "sub-areas" in the North Sea and the data were pooled.

A regression analysis was made between the mercury content and the length, age, and weight of the fish. From the results reported in table 1, it appears that a significant regression was found for the three fishing grounds only between mercury content and age. This relationship is shown in figures 2, 3, and 4.

Although the significant regression between age and mercury content indicates that the age of the fish should be taken into account when deciding upon a specific sampling scheme, these results show that for practical monitoring purposes a wide range of age-groups (e.g. 3 to 12 years) can be considered without decreasing the reliability of the final conclusions.

(*) The results reported here are part of a 5-year study on the mercury content of sole (to be published later)

Table 1 - Regression analysis between mercury content (ppm), length (cm), age (year) and weight (g) of sole (Solea solea) (*).

Area	Length		Age		Weight	
	Equation	r (*)	Equation	r(*)	Equation	r(*)
North Sea	-		$y = 12.2 \cdot 10^{-3} x + 148 \cdot 10^{-3}$	0.349	$y = 26.58 x^{0.339} \cdot 10^{-3}$	0.24
Bristol Channel	$y = 10.6 \cdot 10^{-3} x - 174.10^{-3}$	0.405	$y = 14.3 \cdot 10^{-3} x + 66 \cdot 10^{-3}$	0.527	$y = 1.85 x^{0.755} \cdot 10^{-3}$	0.57
Irish Sea	-		$y = 23.5 \cdot 10^{-3} x + 233 \cdot 10^{-3}$	0.421	-	

(*) r = correlation coefficient; significance 99.9%.

Fig. 1.

Sampling locations of sole (Solea solea) and total Belgian catches (1973)

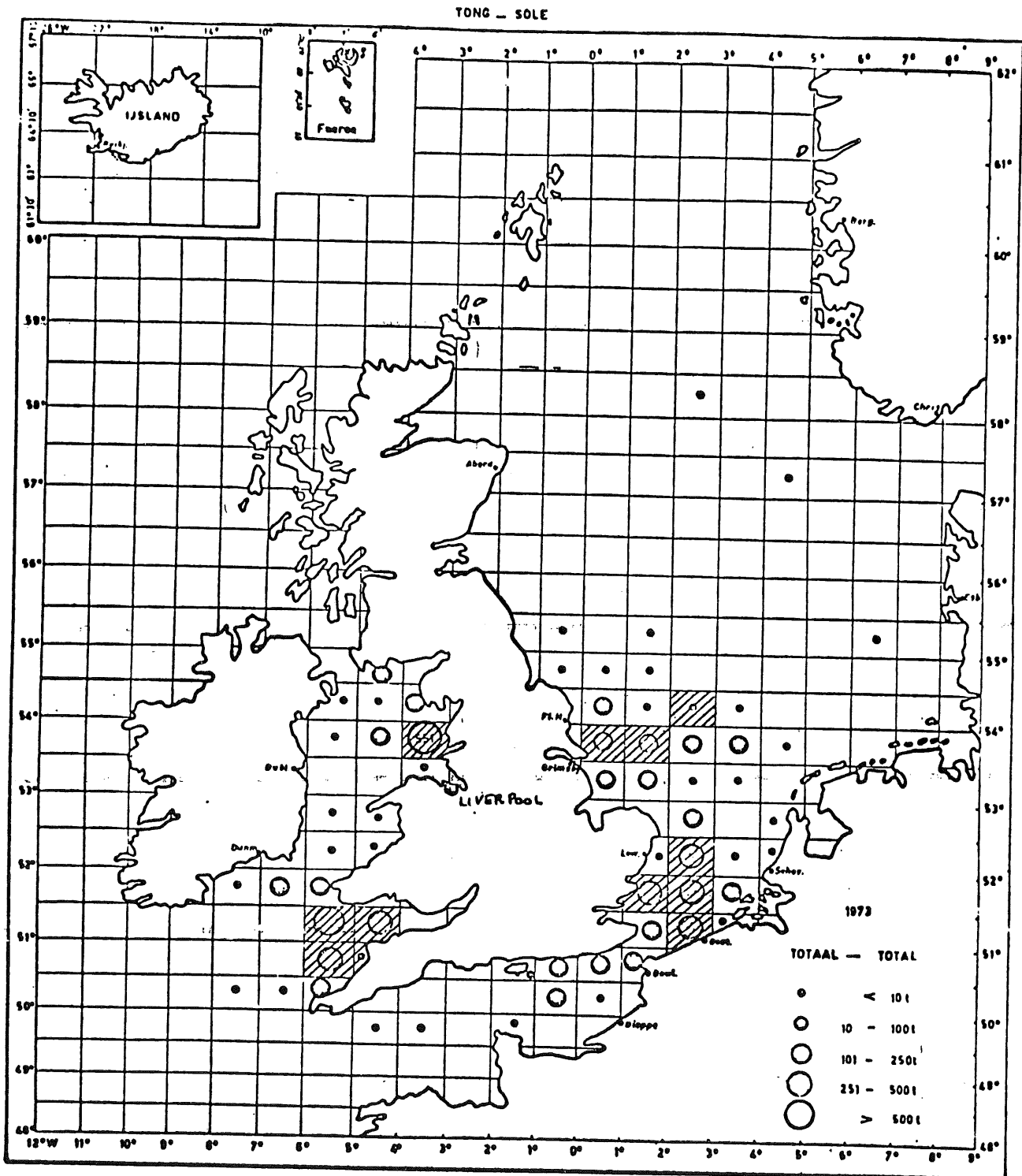


Fig. 2.

Regression between mercury content and age (North Sea)

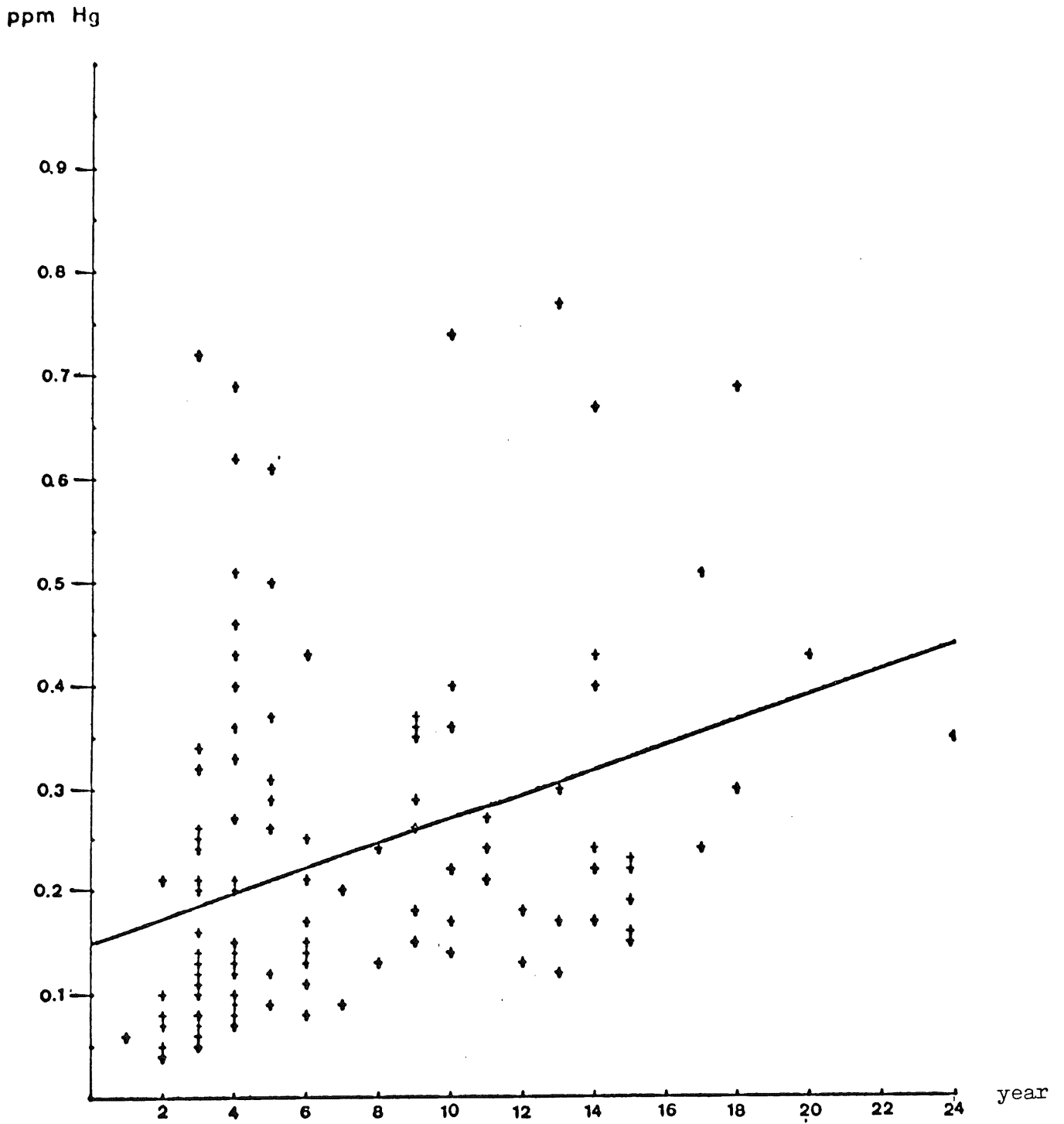


Fig. 3.

Regression between mercury content and age (Bristol Channel)

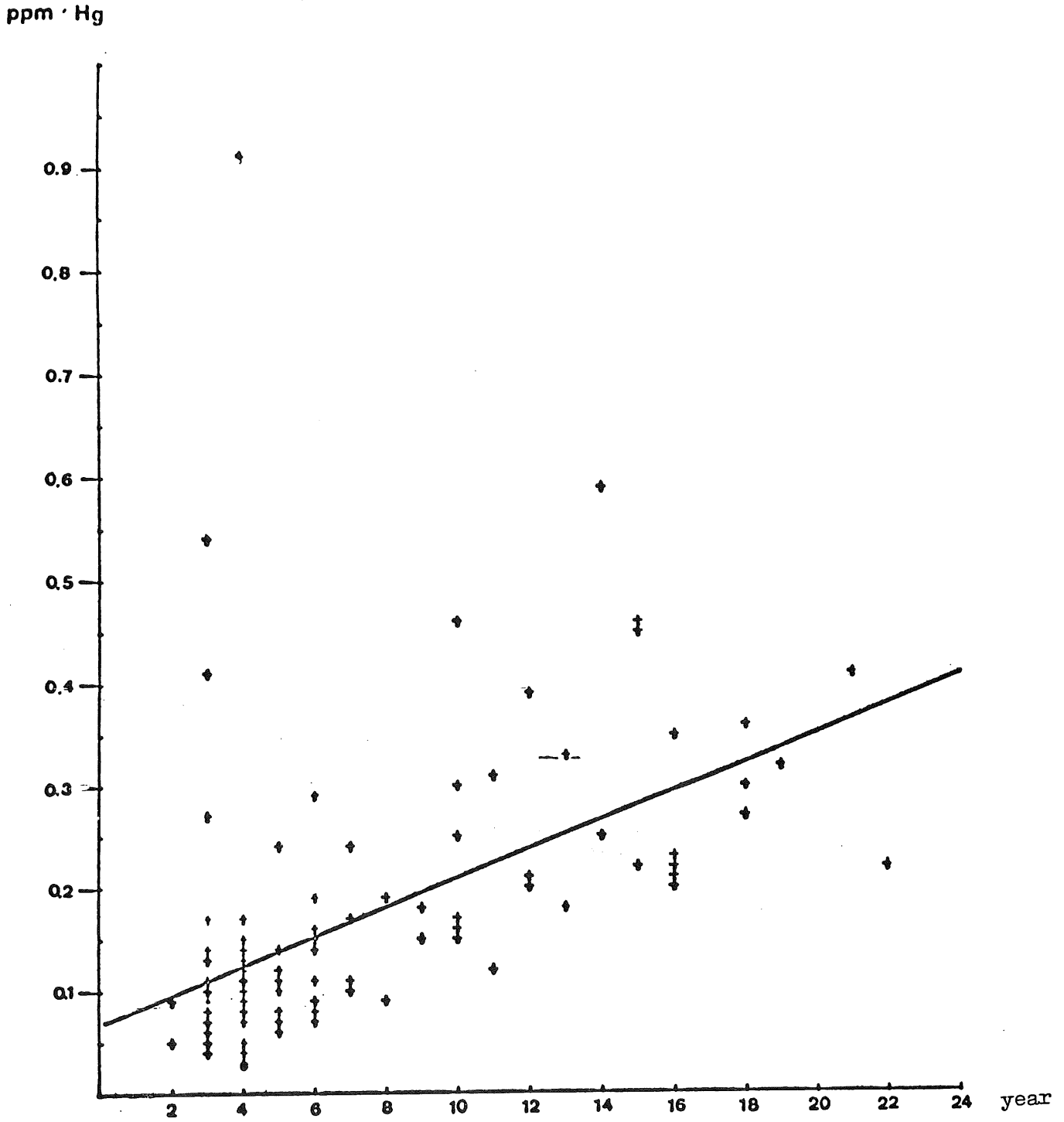
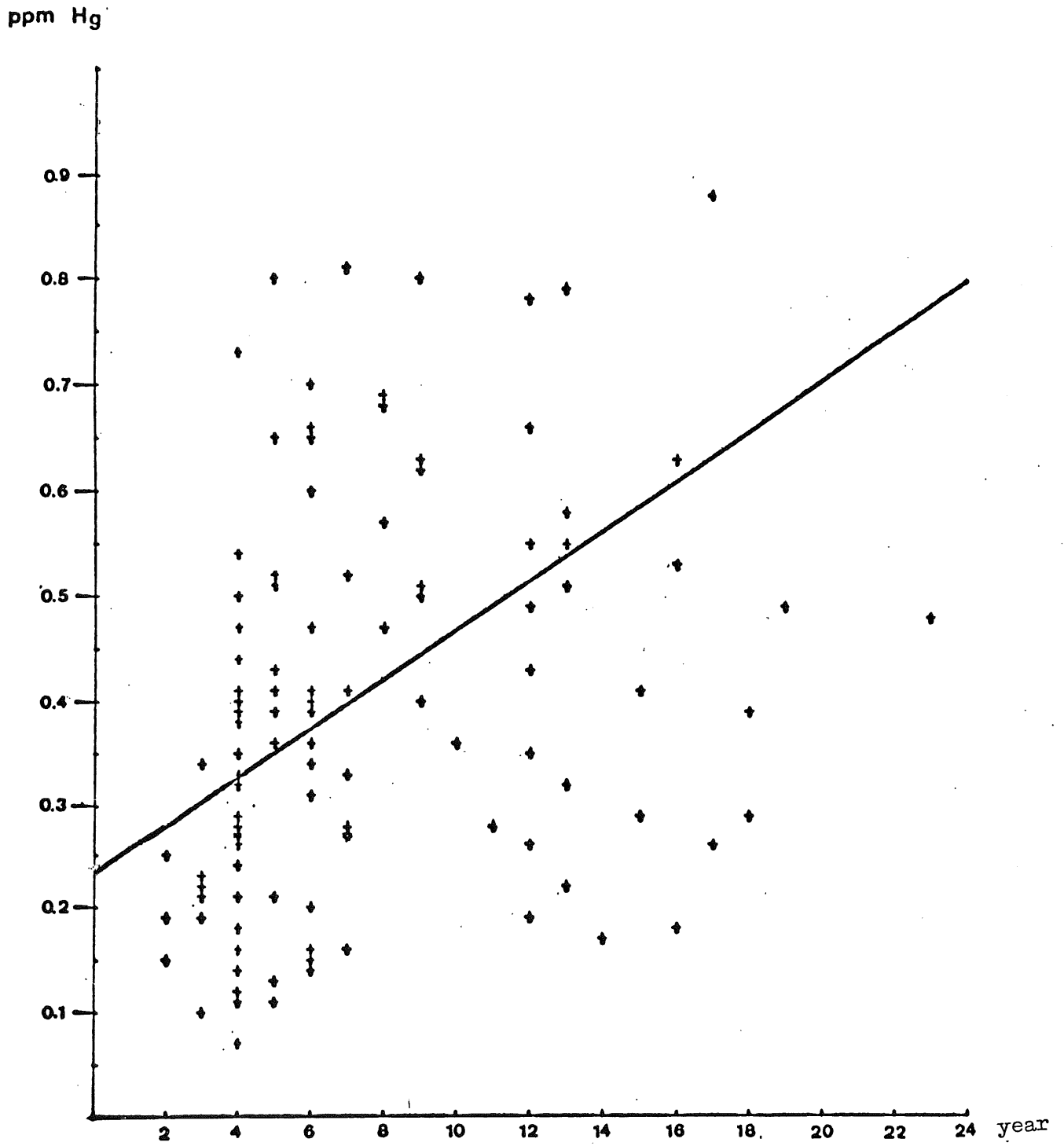


Fig. 4.

Regression between mercury content and age (Irish Sea)



Results of the Regression Analysis Studies

England/Wales

by

Dr J E Portmann (MAFF Burnham-on-Crouch)

In most baseline surveys or monitoring programmes attempts are made to eliminate biological variables by sampling the same size/age animals at the same time of year and where practicable care is taken to ensure that the animals collected are of the same sex. However, just how important it is to do all this is not clear, although adherence to a large number of restrictions or measurement of a large number of variables poses substantial difficulties for the sampling organisation. It was agreed at the 1977 meeting of the Working Group on Marine Pollution Baseline and Monitoring Studies in the North Atlantic, that some work should be done to try and establish to what extent biological variables influence the concentration of pollutants found in the marine organisms.

Description of Work Conducted

In the course of 1977 three fairly large samples of three species of fish were collected partly with this objective in mind. The samples were: 284 specimens of blue whiting from an area northwest of Ireland, 100 flounders from the outer Thames Estuary and 50 cod from the North Sea off Lowestoft. Because the samples were also collected for other purposes it was not possible to carry out the same depth of study on each of the species; nor could all the samples be analysed for all the residues normally determined by the Burnham-on-Crouch Laboratory. Brief details of the analyses conducted and the biological variables measured are given below:-

Blue Whiting The muscle of 284 individuals was analysed for mercury, copper and zinc. In each case the concentrations were calculated on a dry and wet weight basis. The individual fish were sexed, measured for overall length (range 18-41 cm), weighed (range 26-278 g) and aged (range 2-15 years).

Flounders The muscle of 100 individuals was analysed for mercury, copper and zinc, and the livers of some fish were analysed for α and α HCH, dieldrin, DDE, DDD, DDT, and PCBs. The concentrations of metals were calculated on both a wet and dry weight muscle tissue basis and the organochlorine compound concentrations were calculated on a wet liver tissue and extractable fat basis. The individual fish were sexed, measured for overall length (range 16-36 cm), weighed (range 25-500 g) and aged (range 2-8 years).

Cod The muscle of 50 individuals was analysed for mercury, copper and zinc. In each case the concentrations were calculated on both a wet and dry weight basis. The individual fish were all of the same year class, i.e. 1-group, but ranged in weight from 265-890 g and in length from 31 - 45 cm.

Each set of data was subjected to a multiple regression analysis using a stepwise technique which selected the best subset of variables from all those submitted but also calculated the equation involving all variables even if they are not significant. This was done separately for each accumulated substance and as appropriate separately for the concentration in wet or dry weight and wet and fat weight. Since the blue whiting sample was large, the whole process was repeated for male and female blue whiting separately. The number of male flounders was small and so after elimination of the male flounder data the entire process was repeated using only the female flounder data. The cod were not sexed and no further multiple regression analysis was conducted.

In the event it proved that there were very few significant multiple regression equations and the data was therefore also subjected to simple linear regression analysis.

Results

The results of the multiple regression analysis are given in Table 1 and the results of the linear regression analysis are given in Tables 2a, b and c.

The three biological variables length, weight and age are all to some extent interdependent. Since the multiple regression analysis conducted seeks the most important variable first and then moves to the next most important, effecting its inclusion only if the relationship is improved, it might be expected that few full multiple regression equations would be found. In the event this proved to be so and one variable was of predominant importance in most cases (Table 1). In only two cases were all three variables of significance in the equation (zinc in blue whiting and zinc in flounders) and in both cases this applied only to the data evaluated on a wet weight basis, suggesting that although the statistical relationships were different the real significance of the differences might not be so important.

It will be noted that in most cases the value of b_0 in the multiple regression equation is greater for the dry weight relationship by roughly a factor of 5 or 6, i.e. the difference between the wet and dry weights themselves. Since b_0 represents the base level of error in the data this would be expected and the only cases where the relationship did not hold was where the equation was also markedly different, e.g. the flounder mercury data.

No significant multiple regressions were found between the three variables and the concentrations of pesticide and PCB in the liver of flounder, either on a wet or dry weight concentration basis. This indicates that accumulation is independent of length, weight and age and that accumulation is more likely to be related to ambient or recent ambient exposure levels.

In looking at the linear regression data (Tables 2a, 2b, and 2c), where there is a close correlation, the correlation coefficient will be close to unity and the C value will be low indicating that the line passes through the origin. Thus the higher the value of r the more strongly the concentration of accumulated substances is correlated to that variable. Where the multiple regression analysis found only one variable to be of importance then this same variable is also the most strongly correlated in the three linear regression analysis equations: in all cases this proved to be so.

From examination of the blue whiting mercury and copper data, it appears that provided the length of the animals sampled is measured (or for strictly comparable data year to year, fixed) there is no need to age the fish, although for the zinc data both weight and length appear to be equally important. The picture with flounders appears more complex with age being an important variable for mercury affecting accumulation and whereas both length and weight appear to be related to copper and zinc levels. Cod used in this study were all of the same age and with regard to mercury in tissue, weight is the main variable and length can be ignored: copper and zinc concentrations appear to be dependent on both weight and size. Further data will be obtained on this stock of cod as they grow older and this further data may reveal whether age is as important as weight appears to be for mercury, and whether it is more important than either weight or length for copper and zinc.

All the data were examined on both a wet and dry weight basis; this showed, in general, that there was no significant difference in the degree of correlation obtained. This indicates that at least for the three species of fish examined there is unlikely to be any necessity to express the results on a dry weight basis.

The blue whiting data were also examined to see whether **there** was any significant difference between the males and females; any difference would clearly affect the sampling programme. The data showed that with regard to mercury the regression slopes for all three biological variables were significantly higher for the female fish than for the male; for zinc the situation was virtually reversed with the regression slope for data from males being significantly higher except when related to age, where no significant difference was shown. Copper, on the other hand, was not so straightforward, with the regression slope of the data from females being significantly higher for weight, that for males being higher for length and no significant difference being evident between the two sexes with regard to age. As was indicated by the multiple regression analysis no significant linear regressions could be established between variables and the levels of accumulation of pesticide and PCB in liver.

TABLE 1

MULTIPLE REGRESSION EQUATIONS ($Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3$)
 FOR RELATIONSHIP BETWEEN CONCENTRATION OF SUBSTANCE IN TISSUES,
 AND LENGTH, WEIGHT AND AGE OF 3 SELECTED SPECIES.

Y = concentration (mg/kg)

x_1 = length (cm)

x_2 = weight (g)

x_3 = age (yr)

Regression coefficients

SPECIES	Hg (mg/kg)				Cu (mg/kg)				Zn (mg/kg)				Base of Concentration Data (mg/kg)
	b_0	LENGTH	WEIGHT	AGE	b_0	LENGTH	WEIGHT	AGE	b_0	LENGTH	WEIGHT	AGE	
BLUE WHITING	-.234	.0115	N/S	N/S	1.11	-.0208	N/S	N/S	10.26	-.159	-.0195	.364	Wet weight
	-1.52	.0729	N/S	N/S	5.58	-.0926	N/S	N/S	38.98	N/S	-.174	2.21	Dry weight
(FEMALE)	-.262	.0125	N/S	N/S	1.08	-.0195	N/S	N/S	6.65	N/S	-.0117	N/S	Wet weight
	-1.69	.0791	N/S	N/S	5.50	-.0874	N/S	N/S	35.72	N/S	-.0478	N/S	Dry weight
(MALE)	.0238	N/S	N/S	.0080	.852	N/S	-.0035	N/S	8.14	N/S	-.0568	.717	Wet weight
	.116	N/S	N/S	.0497	4.80	N/S	-.0189	N/S	49.03	N/S	-.426	5.32	Dry weight
FLOUNDERS	.0237	N/S	N/S	.0863	1.23	-.030	N/S	N/S	29.72	-1.34	.028	1.55	Wet weight
					6.22	-.152	N/S	N/S	65.16	N/S	-.114	N/S	Dry weight
(FEMALE)	-.060	N/S	N/S	.104	5.72	N/S	-.00076	N/S	12.05	-.450	N/S	1.384	Wet weight
					3.14	N/S	-.0048	N/S	53.31	N/S	-.115	3.027	Dry weight
COD	.165	-.00658	.00028	N/A	.487	N/S	-.00019	N/A	-	N/S	N/S	N/A	Wet weight
	.815	-.0326	.0014	N/A	2.51	N/S	-.00097	N/A	16.18	N/S	.0040	N/A	Dry weight

N/S not significant

N/D no data

N/A not applicable

TABLE 2a

LINEAR REGRESSIONS ($Y = BX + C$) AND CORRELATION CO-EFFICIENTS FOR THE CONCENTRATION OF SUBSTANCES IN TISSUES AND THE LENGTH (CM) OF THOSE SPECIES OF FISH.

SPECIES	Hg (mg/kg)			Cu (mg/kg)			Zn (mg/kg)			Base of Concentration Data (mg/kg)
	B	C	r	B	C	r	B	C	r	
BLUE WHITING	.0122	- .258	.723	-.0169	.988	-.424	- .132	9.54	-.301	Wet weight
	.0762	-1.63	.720	?	:	?	- .449	45.24	-.180	Dry weight
(FEMALE)	.0132	- .289	.717	-.0146	.923	-.369	- .0886	7.63	-.302	Wet weight
	.0822	-1.82	.712	-.0675	4.80	-.347	- .248	36.07	-.149*	Dry weight
(MALE)	.0089	- .168	.532	-.0293	1.32	-.474	.172	2.30	.197*	Wet weight
	.0544	-1.05	.518	-.130	6.54	-.368	1.30	3.69	.239	Dry weight
FLOUNDERS	.0151	- .066	.430	-.0303	1.23	-.682	- .540	20.11	-.630	Wet weight
	.0119	1.56	.086*	-.152	6.22	-.659	-2.20	98.96	-.735	Dry weight
(FEMALE)	.0243	- .339	.439	-.0185	.903	-.566	- .218	10.63	-.242*	Wet weight
	-.108	4.97	-.550	-.107	4.96	-.549	-2.19	97.96	-.672	Dry weight
COD	.0058	- .151	.702	-.00857	.707	-.352	.0308	2.41	.263*	Wet weight
	.0300	- .780	.701	-.0429	3.61	-.339	.181	11.55	.299	Dry weight

* regression not significant

There were no significant linear regression relationships for the O/C and PCB in Flounder data with any of the three variables, age, length or weight.

TABLE 2b

LINEAR REGRESSIONS ($Y = BX + C$) AND CORRELATION CO-EFFICIENTS FOR THE CONCENTRATION OF SUBSTANCES IN TISSUES AND THE WEIGHT (g) OF THESE SPECIES OF FISH.

SPECIES	Hg (mg/kg)			Cu (mg/kg)			Zn (mg/kg)			Base of Concentration Data (mg/kg)
	B	C	r	B	C	r	B	C	r	
BLUE WHITING	.0010	-.021	.659	-.0015	.678	-.424	-.0133	7.29	-.336	Wet weight
	.0061	-.127	.631	-.0074	3.74	-.393	-.0585	39.25	-.256	Dry weight
(FEMALE)	.00102	-.0197	.627	-.0013	.650	-.372	-.0086	6.08	-.332	Wet weight
	.00605	-.106	.593	-.0060	3.54	-.351	-.0363	33.43	-.247	Dry weight
(MALE)	.00011	.064	.285	-.00018	.530	-.126*	-.00202	6.81	.098*	Wet weight
	.00067	.378	.271	-.00079	3.04	-.095*	.0147	37.83	.115*	Dry weight
FLOUNDERS	.00055	.204	.309	-.00139	.733	-.614	-.0237	11.12	-.544	Wet weight
	-.00051	1.94	-.072*	-.00734	3.79	-.623	-.114	65.16	-.745	Dry weight
(FEMALE)	.00047	.211	.212*	-.00076	.572	-.573	-.00670	6.42	-.183*	Wet weight
	-.00483	3.15	-.609	-.00480	3.14	-.608	-.0945	59.81	-.714	Dry weight
COD	.00014	-.0088	.777	-.00019	.488	-.359	.00071	3.18	.278	Wet weight
	.00072	-.0443	.773	-.00098	2.52	-.354	.00408	16.15	.308	Dry weight

* regression not significant

TABLE 2c

LINEAR REGRESSIONS ($Y = BX + C$) AND CORRELATION CO-EFFICIENTS FOR THE CONCENTRATION OF SUBSTANCES IN TISSUES AND THE AGE (YR) OF THESE SELECTED SPECIES OF FISH.

SPECIES	Hg (mg/kg)			Cu (mg/kg)			Zn (mg/kg)			Base of Concentration Data (mg/kg)
	B	C	r	B	C	r	B	C	r	
BLUE WHITING	.0147	.0019	.665	-.0270	0.68	-.516	-.055	6.21	-.093*	Wet weight
	.0931	-.026	.672	-.0991	3.56	-.345	.156	31.86	.046*	Dry weight
(FEMALE)	.0174	-.0064	.688	-.0259	.671	-.509	-.140	6.12	-.317	Wet weight
	.110	-.0773	.693	-.0887	3.47	-.306	-.307	31.49	-.127*	Dry weight
(MALE)	.0086	.0202	.623	-.0275	.683	-.487	.263	5.34	.357	Wet weight
	.0545	.0926	.632	-.112	3.64	-.364	2.03	26.55	-.455	Dry weight
FLOUNDERS	.0863	.0237	.551	-.101	.871	-.510	-.142	7.55	-.179*	Wet weight
	-.0012	1.86	-.002*	-.496	4.12	-.481	-6.92	67.72	-.517	Dry weight
(FEMALE)	.104	-.0602	.516	-.0526	.593	-.441	.0954	4.39	.029*	Wet weight
	-.260	3.01	-.365	-.0254	2.18	-.157*	-3.14	50.10	-.264	Dry weight

* regression not significant

Annex VI

PROCEDURES TO BE FOLLOWED FOR SAMPLING AND PREPARATION OF FISH AND SHELLFISH
TO BE USED IN MONITORING PROGRAMMES IN THE NORTH ATLANTIC AREA¹⁾

1. Samples of fish or shellfish which are collected in connection with programmes designed primarily to assess the risk to human health are not always suitable for the detection of trends in either space or time, other than on a broad scale. In a public health context no set limits are suggested since it is obviously necessary for the samples collected to reflect the commercial landings and these will vary from site to site and country to country. However, as far as practicable, samples should be collected at the same time of year, and in the same conditions.
2. Multiple regression analysis of pollutant concentrations in relation to several physiological factors shows that in order to be able to establish small changes in pollutant concentrations with time or space, it is necessary also to have full information on the various physiological factors which can have an influence on the concentration of a pollutant found in an organism.
3. Thus, in order to be able to use samples of fish which are collected for human health risk assessment purposes for trend assessment as well, it is necessary to analyse the organisms individually and to determine their age, length, weight, and sex whenever possible and to calculate the condition factor and somatic liver index, so as to allow for a multiple regression analysis.
4. It may be possible to use fish samples without collecting information on all these variables, for any particular species, or pollutant, or area, provided that a thorough investigation is first made using multiple regression techniques to establish the critical variables. Once this investigation has been completed, it should be possible to standardise the critical variable(s). However, it is likely that this will have to be done separately for each species, pollutant and area, as different variables are likely to be significant in different cases.
5. Areas from which samples are to be taken will be those which countries have designated to the Secretariat of the Oslo and Paris Commissions²⁾.

1) The footnotes found in these Procedures contain special instructions for laboratories participating in the ICES Coordinated Monitoring Programme and may be ignored by other laboratories.

2) Areas of special interest are the entire Irish Sea; the German Bight and Southern Bight of the North Sea; the estuaries of the Thames, Forth, Rhine, Schelde and Clyde; the Skagerrak, Kattegat, and Oslofjord; and certain parts of the Gulf of St Lawrence and the New York Bight.

6. Species on which sampling should be concentrated are mussels (Mytilus edulis), shrimp (Crangon crangon), and two of the following fish species: flounder (Pleuronectes flesus) or plaice (Pleuronectes platessa) and mackerel (Scomber scombrus or Scomber japonicus) and cod (Gadus morhua) or hake (Merluccius merluccius)³⁾.
7. All samples should, wherever possible, be collected in a manner which is similar in successive years for the particular species and sampling site.
8. All fish samples should be collected ungutted and preserved (deep frozen) as soon as practicable after collection, and length, weight and age should be determined before deep-freezing, if possible.
9. A sample of fish should consist of 20, or at least 10, individual specimens; if possible, each individual should be analysed separately. If this is not practicable, an equal-sized portion should be taken from each fish and the mixture should be thoroughly homogenised and the analysis done in duplicate. Details on sample preparation for analysis are given in Appendix I.
10. For environmental trend assessment, the muscle and liver of fish and the soft tissue of mussels should be analysed. For human health risk assessment, it is only necessary to analyse the muscle tissue of fish, in homogenised samples if more convenient, the peeled tail of shrimps and the soft tissue of mussels.
11. Mussels should be held in clean (settled) sea water from the area of collection for 12 - 24 hours to allow discharge of adventitious silt material as pseudofaeces. Maximum length, regardless of orientation, should be measured for each specimen.
12. A mussel sample should consist of at least 50 individuals and, after cleansing and measuring as described in 11 above, the individual animals should be carefully recovered from their shells and placed together in a beaker. The supernatant shell liquor should be decanted and the residual body meats may then be preserved (deep-frozen) or prepared for analysis.
13. A sample of shrimps should consist of at least 100 individuals and should be prepared for analysis by boiling in sea water, from the area of collection, for 10 minutes. The tails should then be removed, peeled and thoroughly homogenised in preparation for analysis. (This process assumes that the reason for selection of shrimps was purely for human health assessment, since a wide range of factors, not all of which are mentioned in 3 above, can affect the total body burden of a pollutant in shrimps.)
14. Pollutants to be analysed in particular are mercury, cadmium and PCBs⁴⁾.

3) In addition to the species named above, species also of value are herring (Clupea harengus), pilchard or sardine (Sardina pilchardus), and sole (Solea solea or Solea senegalensis).

4) Additional pollutants of special interest are copper, zinc, arsenic and selenium, HCB and organochlorine pesticides. Data on other substances may be included at the discretion of the individual participants.

15. All results of analyses of fish and shrimps for metals are to be reported on a wet weight basis and preferably also on a dry weight basis. All results of analyses of mussels for metals are to be reported on a dry weight basis. All results of analyses for organochlorine compounds must be reported on both a wet weight basis and on an extracted fat weight basis, or as a minimum be accompanied by a fat weight determination result.
16. Dry weight determinations should be carried out in duplicate by air-drying to constant weight at 105°C of sub-samples of the material analysed for the pollutants.
17. Fat weight should be determined on a sub-sample of the extract used for the organochlorine compound analyses. The results should be accompanied by a brief description of the method used for extraction.
18. In all cases, full results of all analyses performed should be provided, i.e., individual data, geometric mean (assuming log-normal distribution of data) and standard deviations, together with full details of the site, date and method of collection, preservation details (if appropriate), date of analysis and brief details of the methods used. In the case of the results for PCBs, the formulation used for the quantification should be stated, and examples of typical standards and PCB chromatograms should be provided.
19. Analytical results should be submitted on an individual laboratory basis from laboratories which have recently taken part in an ICES intercalibration exercise. The name of the laboratory should be provided together with the details of the intercalibration exercises⁵⁾.

5) For laboratories participating also in the ICES Coordinated Monitoring Programme, data should be reported also to the Environment Officer (with a copy to the Chairman of the fish sub-group) not later than 31 March of the calendar year following that in which samples were collected.

Annex VI, Appendix I

PREPARATION OF SAMPLES FOR ANALYSIS

1. When directly handling tissues to be used in analysis of harmful substances, all sources of possible contamination should be avoided. For material to be used for heavy metal analysis, direct contact with metallic substances should be avoided. Similarly, material to be used for analysis for organochlorines should not be placed in direct contact with plastics. As a general rule, the contact time between the sample and the tools should be kept as short as possible. Grinding procedures should be checked very critically to make certain that no contamination can occur and in removing the tissue and placing it in containers, it is very important to use appropriate materials, tools and storage containers.
2. To prepare material for metal analysis, cut or crushed pieces of glass should be used except for Hg analysis, in which case acid-resistant stainless steel scalpels may be used. Homogenisation and grinding of deep frozen material should be carried out using materials of silica or Teflon.
3. For organochlorine analysis the material may be cut with acid-resistant steel scalpels and homogenised using Ultra Turrax type homogenisers. All the tools used must be thoroughly washed and cleaned to prevent sample contamination.
4. The dissection room should be kept clean and the air should be freed from particles as much as possible. It is an advantage if the work can be carried out in a hood or under some shelter in order to prevent a direct fall-out of particles onto the sample. The dissection should always be carried out on a clean glass plate with the appropriate tools for each type of contaminant.
5. The dissection of fish is easiest when the material is half frozen, at least concerning the surface layers of muscle tissue. For dissection of other organs, the thawing must proceed further, but it is an advantage if, for example, the liver is still frozen, as the loss of liquid and fat when cutting the tissue makes the determination of dry weight and fat content less accurate.
6. The epidermis and subcutaneous tissue should be carefully removed from the fish. Samples should be taken under the red muscle layer. In order to ensure uniformity of samples, the right side dorso-lateral muscle should be taken as the sample. If possible the entire right dorsal lateral filet should be used as a uniform sample, from which sub-samples can be taken after homogenising for dry weight, heavy metal and organochlorine determinations. If, however, the amount of material so obtained would be too large a sample, a specific portion of the dorsal musculature should be chosen for the sample. It is recommended that the portion of the muscle lying directly under the first dorsal fin or, in flounder, plaice, and sole, the central part of the filet, be utilised in this case. As both fat and water content vary significantly in the muscle tissue from the anterior to the caudal muscle of the fish, it is important to obtain the same portion of the muscle tissue for each sample to ensure comparability of samples.
7. After muscle preparation, the liver should be completely and carefully removed while still partly frozen to avoid water and fat loss. Immediately after removing it from the fish, the liver should be returned to the freezer so that it will be completely frozen prior to further handling. This is particularly important for cod liver.

8. The whole soft body of the mussels including the adductor muscle should be carefully removed and combined with the others to be included in the sample. Care should be taken to avoid excessive tissue damage and thus cell water loss during this procedure. After placing all the animals in a beaker, the supernatant shell liquor should be decanted and the whole sample kept deep frozen pending analysis.
9. If the sample is to be used for trend analysis, for each fish the total body weight in grams should be recorded, as well as the total length (length between the nose tip and the tip of the tail) in centimetres. The sex should be indicated if at all possible. The age should be determined and should generally be given according to the number of annual rings on the scales or otoliths.
10. The weight of the liver should be recorded in grams. Obtaining the correct liver weight can depend on using the appropriate procedures. The complete liver should be removed very carefully during dissection of the partly-thawed specimen. This should be done by a person skilled in the technique to ensure that the full sample is obtained.
11. As a measure of physiological condition, the Liver Somatic Index and the Condition Factor should be calculated for each fish.

The Liver Somatic Index is obtained by

$$\frac{\text{weight of liver}}{\text{total weight of fish}}$$

The Condition Factor of the fish is calculated as

$$\frac{w \times 10^5}{l^3}$$

where w (in gm) is the total body weight, and l (in mm) is the standard length (the length between the nose tip and the last caudal vertebra).

Annex VII

PRELIMINARY REPORT ON THE FOURTH ICES TRACE METAL INTERCOMPARISON EXERCISE FOR CADMIUM AND LEAD ONLY

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INTRODUCTION

At the third meeting of the ICES Working Group on Marine Pollution Baseline and Monitoring Studies in the North Atlantic held in May 1977, it was agreed that a further intercomparison exercise for cadmium and lead should be conducted before the next meeting of the Working Group in May 1978. The Marine Laboratory, Aberdeen, was asked to coordinate this exercise, which was carried out during Sept.-Dec. 1977. This report discussed the results.

DISTRIBUTION OF REFERENCE MATERIAL

In June 1977, 20 gm of fish flour (the same material used in the third ICES trace metal intercalibration exercise) was sent to 18 laboratories in Europe, USA, and Canada with instructions to analyse the sample for lead and cadmium only and return the results by 1 November 1977. The participants were also asked to provide details of their methodology and a description of their instrumentation, and to calculate detection limits for each metal.

RESULTS

The twelve participants in this exercise are given in Table 1. A summary table of the analytical procedures and instrumentation is presented in the appendix.

LEAD

Mean concentrations of lead in the fish flour reported by the twelve laboratories ranged from 0.163 ppm to 3.078 ppm. The means, standard deviations and coefficients of variation are given in Table 1. Four laboratories (University of Connecticut, Belgium, Ireland, and Netherlands) recorded mean concentrations which were much higher than those of the other laboratories and two laboratories (France and Norway) recorded lower means. The internal consistency of the results from five of these six laboratories was high, four of them having coefficients of variation of less than 10%. The mean values from the remaining six laboratories (in reality seven sets of analyses since Germany submitted two sets of data) ranged from 0.507 to 0.682; the maximum, minimum, and median results for each of these seven sets of data are shown in Fig. 1. It is clear that the within-laboratory variability for these six laboratories is not the same throughout. In particular the variability for one laboratory (Canada, MAF) was much higher than for the others, owing largely to the presence of a

single outlying value (1.18 ppm). The variability in the data from the remaining five laboratories was more consistent. The differences in the mean values of these five laboratories may therefore be compared using a standard analysis of variance. This test showed that there were significant differences between the six sets of data and a multiple range test was computed to quantify the differences. The results are summarised below.

Germany(a)	Maryland USA	Connecticut USA	Germany(b)	Scotland	Canada (F&M)
0.507	0.512	0.599	0.628	0.660	0.665

It is interesting to note that the means of the two sets of data supplied by Germany (resulting from two different methods of analysis) differ significantly, set (b), the "extraction" method (see Appendix) giving the higher results.

CADMIUM

Mean concentrations of cadmium in the fish flour reported by the twelve laboratories ranged from 0.022 ppm to 0.208 ppm. Two laboratories (University of Connecticut and National Marine Fisheries Service, Connecticut) recorded means which were much higher than the rest. The results from the University of Connecticut were consistent, but in the results from the National Marine Fisheries Service, Connecticut, there was an outlying value which led to a relatively high coefficient of variation. The maximum, minimum and median results for the remaining ten laboratories are shown in Figure 2.

Within-laboratory variability differed widely between laboratories. This is further illustrated by the coefficients of variation given in Table 1. Belgium quoted results of the analysis to one significant figure only, hence masking any variability in the determinations made. Three laboratories with coefficients of variation of less than 10% recorded rather different mean levels of cadmium in the fish flour sample - Canada (Fisheries and Marine) 0.030 ppm, Germany (set b) 0.022 ppm and Norway 0.051 ppm. The large differences in within-laboratory variability referred to above make it impossible to carry out a reliable analysis of variance to test differences between the mean values.

DISCUSSION

It was generally agreed at the third meeting of the ICES Working Group that the results for cadmium and lead from the third ICES exercise were relatively poor because the majority of participants were using analytical procedures with poor detection limits. The laboratories were asked to review their methodology with a view to improving them. An examination of the methodology used in the current exercise (Appendix) shows that nearly 50% of the laboratories who participated in both exercises have responded to this suggestion. A comparison of the two sets of data for 1975 and 1977 for cadmium and lead (Table 2) and the reported detection limits (Table 3) shows that those laboratories which made major modifications to their analytical procedures recorded lower mean values for cadmium and lead and reported improved detection limits. Two laboratories which did not make the suggested changes to their procedure produced values for cadmium and lead which were similar to those reported by them in the third exercise. The overall mean values for lead and cadmium in this exercise are lower than the overall means obtained during the third exercise (Table 2).

Although the statistical analyses described above show that there are significant differences between the data from individual laboratories, it is clear that the group is progressing towards the production of comparable data for these two metals.

Table 1 Results of Fish Flour Analysis ($\mu\text{g}/\text{gm}$)

LABORATORY	Lead			Cadmium		
	mean	standard deviation	coeff. of variation	mean	standard deviation	coeff. of variation
Belgium	1.505	0.02588	1.7	0.06	-	-
Canada (F&M)	0.665	0.16141	24.3	0.030	0.001643	5.5
Canada (MAF)	0.682	0.25810	37.8	0.058	0.008313	14.3
France*	0.163	0.01211	7.4	0.040	0.008944	22.4
Germany a)**	0.507	0.03830	7.6	0.024	0.004889	20.4
b)	0.628	0.06113	9.7	0.022	0.001722	7.8
Ireland	1.242	0.30311	24.3	0.058	0.010675	18.2
Netherlands	0.871	0.06871	7.9	0.030	0.005907	19.7
Norway	0.297	0.05854	19.5	0.051	0.003601	7.1
Scotland	0.660	0.07348	11.1	0.048	0.009347	19.5
USA (Maryland)	0.512	0.12465	24.3	0.032	0.014516	45.4
USA (Connecticut)	0.599	0.04051	6.8	0.139	0.034440	24.8
USA (University of Connecticut)	3.078	0.09704	3.2	0.208	0.000303	10.1

* Following circulation of this report France has submitted additional analytical data for lead - 0.40, 0.64, and 0.70 ppm; mean value of 0.58 ppm

** See Appendix for difference between (a) and (b)

Table 2 Comparison of Lead and Cadmium Analytical Data ($\mu\text{g}/\text{gm}$) for 1975 and 1977

LABORATORY	LEAD				CADMIUM			
	1975		1977		1975		1977	
	Mean	C.V.*	Mean	C.V.	Mean	C.V.	Mean	C.V.
BELGIUM	2.08	3.7	1.51	1.7	0.053	40.8	0.06	-
CANADA (F&M)	0.52	2.7	0.66	24.3	0.023	12.2	0.030	5.5
CANADA (MAF)	0.25	26.5	0.68	37.8	0.177	33.3	0.058	14.3
FRANCE	4.0	10.0	0.16 ⁺	7.4	0.41	12.2	0.040	22.4
GERMANY	0.53	10.5	0.51	7.6	0.028	6.4	0.024	20.4
NETHERLANDS	0.51	16.6	0.87	7.9	0.055	21.1	0.030	19.7
NORWAY	0.81	2.5	0.30	19.7	0.042	13.3	0.051	7.1
SCOTLAND	0.34	20.5	0.66	11.0	<0.03	-	0.048	19.5
USA - NOAA (Maryland)	1.18	18.0	0.51	24.3	0.17	69.9	0.032	45.4
USA - NOAA (Connecticut)	3.00	45.1	0.60	6.8	<0.24	-	0.139	24.8
USA (Univ. of Connecticut)	2.30	2.8	3.08	3.2	0.39	12.0	0.208	0.1
Overall Mean values	1.50		0.87 0.60**		0.159		0.07 0.043 ⁺⁺	

*C.V. = coefficient of variation

+ Following circulation of this report France has submitted additional analytical data for lead - 0.40, 0.64, and 0.70 ppm; mean value of 0.58 ppm.

** This value has been calculated by excluding the mean values from Belgium and USA (Univ. of Connecticut) and replacing the original French data with the latest set of data.

++ This value has been calculated by excluding the mean values from USA-NOAA (Connecticut) and USA (Univ. of Connect.)

Table 3 Comparison of reported detection limits ($\mu\text{g}/\text{gm}$) for Lead and Cadmium analysis for 1975 and 1977

Laboratory	LEAD		CADMIUM	
	1975	1977	1975	1977
BELGIUM	1	0.05	0.02	0.005
CANADA (F&M)	0.02	0.001	0.005	0.005
CANADA (MAF)	0.007	0.01	0.006	0.005
FRANCE	1.5	0.1	0.05	0.02
GERMANY	0.004	(a) 0.006 (b) 0.005	0.001	(a) 0.0012 (b) 0.0004
NETHERLANDS	0.02	0.018	0.0027	0.008
NORWAY	0.05	0.02	0.005	0.003
SCOTLAND	0.2	0.13	0.03	0.013
USA - NOAA (Maryland)	0.2	0.12	0.015	0.007
USA - NOAA (Connecticut)	1.5	0.067	0.20	0.014
USA (University of Connecticut)	0.35	0.42	0.06	0.04

Appendix

Summary of the analytical procedures and instrumentation used by participants in 4th ICES trace metal intercomparison exercise.

<u>LABORATORY</u>	<u>PROCEDURE</u>	<u>INSTRUMENTATION</u>
BELGIUM	Lead and Cadmium - 1.5 g was dry ashed @ 450°C. Residue dissolved in 2.5 ml HNO ₃ (+ 1 ml H ₂ O ₂). Diluted to 50 ml. 10 μl used for injection.	Perkin Elmer 303 +H.G.A.
CANADA (F&M)	Lead - 0.5 g digested with 10 ml conc HNO ₃ @ 80°C - 90°C. Final volume = 20 ml (50% HNO ₃). Standard addition technique (10 μl sample + 10 μl standard).	Perkin Elmer - 370A and H.G.A. 2100. Deuterium background.
	Cadmium - 0.5 g digested with 5 ml conc HNO ₃ @ 80°C - 90°C. Final volume = 10 ml. Standard addition technique (10 μl sample + 10 μl Standard).	Perkin Elmer - 403 H.G.A. 74 and H.G.A. 2100.
CANADA (MAF)	Lead and Cadmium - 1 g digested with 10 ml conc HNO ₃ @ 100°C. Reduced volume to 1 ml, final volume 10 ml (0.8N HNO ₃). 5 μl used for injection.	Techtron - AA5, carbon rod model 63. Background correction BC-6.
FRANCE	Lead and Cadmium - 1-3 g digested with conc HNO ₃ and H ₂ SO ₄ @ 140°C. H ₂ O ₂ added to eliminate HNO ₃ . Diluted to 100 ml. Standard addition technique 15 μl used for injection.	1L 152 - graphite furnace. Deuterium background correction
GERMANY (a)	Lead and Cadmium - 1 g digested with 15 ml conc HNO ₃ and 3 ml HClO ₄ . HNO ₃ distilled off and final volume made up to 50 ml. Standard addition technique - 50 μl injected.	Perkin Elmer 420 and H.G.A. 76. Deuterium background
	(b) As above but aqueous sample was extracted with dithizone/toluene. Re-extracted from organic phase using 0.5N HCl. 50 μl of this solution was then injected.	"
NETHERLANDS	Lead - 0.2 g was digested with 5 ml HNO ₃ (70%). Volume reduced to 0.5 ml and diluted to 10 ml. Standard addition technique - (2 μl sample + 6 μl standard).	Varian Techtron No 1100 and CRA 63. Background correction - BC-6.
	Cadmium - as above but final solution made up in 0.1 N H ₂ SO ₄ .	"

<u>LABORATORY</u>	<u>PROCEDURE</u>	<u>INSTRUMENTATION</u>
NORWAY	Lead and Cadmium - 0.15 g was digested with 2 ml HNO_3 and 2 ml HClO_4 @ 110°C , diluted to 10 ml. 42 ml aliquot evaporated to dryness, taken up in 0.4 ml HNO_3 (5%). Standard addition technique.	Perkin Elmer 403 and HGA - 76. Deuterium background
SCOTLAND	Lead and Cadmium - 2.0 g digested with 20 ml conc HNO_3 - evaporated to 5 ml and then diluted to 25 ml. Standard addition technique (20 μl sample).	Perkin Elmer 603 and HGA - 76. Deuterium background.
USA - NOAA Maryland	Lead and Cadmium - 0.1 gm was digested with 0.75 ml (24:24:1:: HNO_3 , HClO_4 , H_2SO_4) @ 300°C . The sample was buffered with 5 ml of 1M sodium acetate / 0.2M sodium chloride. Plated @ 1000 mV for 30 mins, stripped @ 60 mV/sec.	ASV - Environmental Sciences Associated Model 2014
USA-NOAA Connecticut	Lead and Cadmium - 1 g was digested with 5 ml conc HNO_3 @ 130°C - 140°C . Evaporated to dryness. Final volume made up to 25 ml (HNO_3 -5%)	Perkin Elmer - 305 and HGA - 2100.
USA - University of Connecticut	Lead and Cadmium - 1.6 g was digested with 10 ml HNO_3 @ 50°C . Final volume 25 ml.	1L 151 (flame) + deuterium background.
IRELAND	Lead and Cadmium - 1 gm dry ashed @ 450°C . Residue dissolved in 20 ml HNO_3 , diluted to 25 ml.	Perkin Elmer HGA 76

